

Pázmány Péter Katolikus Egyetem
Információs Technológiai és Bionikai Kar

A mitokondrium anyagcseréjének modellezése

Sváb Gergely

Info-bionikus mérnök
I. évfolyam

Konzulensek:

Dr. Szederkényi Gábor, egyetemi tanár
Pázmány Péter Katolikus Egyetem
Információs Technológiai és Bionikai Kar

Dr. Tretter László, egyetemi tanár
Semmelweis Egyetem
Orvosi Biokémiai Intézet

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Statement

Alulírott Sváb Gergely, a Pázmány Péter Katolikus Egyetem Információs Technológiai és Bionikai Karának hallgatója kijelentem, hogy ezt az TDK dolgozatot meg nem engedett segítség nélkül, saját magam készítettem, és a beszámolóban csak a megadott forrásokat használtam fel.

Minden olyan részt, melyet szó szerint, vagy azonos értelemben, de átfogalmazva más forrásból átvettem, egyértelműen a forrás megadásával megjelöltem. Ezt az TDK dolgozatot más szakon még nem nyújtottam be.

Budapest, 2017. november 16.

Sváb Gergely

Abstract

Mitochondria are cell organelles with prokaryotic origin that established endosymbiosis with ancient eukaryotic cells during evolution. Mitochondria produce energy during the catabolic degradation of carbohydrates, fats, proteins and nucleic acids. Additionally, molecules of the mitochondrial metabolism take part as precursors in the construction of the anabolic procedures [1].

Molecules involved in the citric acid cycle can transform into each other depending on the current metabolic status of mitochondria. Regulation of citric acid cycle depends on the concentration of intermediary molecules and the changes in redox potential. As a result of these reactions so-called reducing equivalents are produced, which can transport electrons to the electron transport chain, where the electrons' energy plays an important role in the synthesis of ATP which can later be used as an energy source in other cell functions.

In the Neurobiochemistry working group (Institute of Medical Biochemistry, Semmelweis University), we have taken enzyme kinetic measurements of several mitochondrial enzymes, and we have determined and analyzed interactions between enzymes and substrates. We have measured reactive oxygen species productions and the mitochondrial O_2 consumptions with different respiratory substrates [2, 3, 4, 5]. The need naturally arises for further analysis of the process. Therefore, a suitable quantitative (mathematical) model is constructed which describes the temporal changes in the quantities of citric acid cycle's molecules. The modeling goal in this research phase is to describe qualitatively the increased/decreased operation of the catalyzing enzymes as well as the modified operation of the intermediate molecules. In next phase we would like to be able to predict whether mitochondria have the ability to adapt to this new metabolic state by activating/inhibiting other metabolic pathways or the metabolism will become pathological. In previous studies similar models [6, 7, 8, 9, 10] were constructed, it falls under our goals to promote further development compared to the known models, to combine these to each other and to expand them in other aspects.

Abbreviations, symbols

AcCoA	Acetyl-coenzyme A
ACON	Aconitase
ADP	Adenosine diphosphate
AGC	Aspartate glutamate carrier
ASAT	Aspartate aminotransferase (=GOT)
Asp	Aspartate
ATP	Adenosine triphosphate
α KG	α -ketoglutarate
α KGDH	α -ketoglutarate dehydrogenase
α KGT	α -ketoglutarate transporter
CAC	Citric acid cycle
CPS	Citrate-pyruvate shuttle
Cit	Citrate
CoA-SH	Coenzyme A
CS	Citrate syntase
CTP	Citrate transporter
Cytc	Cytochrome c
DIC	Dicarboxylate carrier
E	Enzyme
ES	Enzyme-substrate complex
ETC	Electron transport chain
FAD	Flavin adenine dinucleotide (oxidized form)
FADH ₂	Flavin adenine dinucleotide (reduced form)
Fum	Fumarate
FH	Fumarase (fumarate hydratase)
GC	Glutamate carrier
GDP	Guanosine diphosphate
Glu	Glutamate
GOT	Glutamate oxaloacetate transaminase (=ASAT)
GTP	Guanosine triphosphate
IDH	Isocitrate dehydrogenase

ICit	Isocitrate
K_M	Michaelis Menten constant
Mal	Malate
MAS	Malate-aspartate shuttle
MDH-c	Malate dehydrogenase (cytoplasmic isozyme)
MDH-m	Malate dehydrogenase (mitochondrial isozyme)
ME	Malic enzyme
NAD^+	Nicotinamide adenine dinucleotide (oxidized form)
NADH	Nicotinamide adenine dinucleotide (reduced form)
NADP^+	Nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
OA	Oxaloacetate
P	Phosphate, H_2PO_4^-
PC	Pyruvate carboxylase
PDH	Pyruvate dehydrogenase
PEP	Phosphoenolpyruvate
PT	Pyruvate transporter
Pyr	Pyruvate
ROS	Reactive oxygen species
$S_{n \times k}$	Stoichiometric matrix
SDH	Succinate dehydrogenase
SucCoA	Succinyl coenzyme A
SCS	Succinyl coenzyme A synthetase
Suc	Succinate
UCP	Uncoupling protein
UQ	Ubiquinon
UQH_2	Ubiquinol
V_{max}	Maximum reaction rate
V_n	Reaction rate vector

Chapter 1

Introduction

1.1 Mitochondrion

Mitochondria are organelles in cells with prokaryotic origin. These organelles produced by endosymbiosis with eukaryotic cells during evolution [11]. Mitochondria are very important, because they take part in the bioenergetic functions of the cells, control metabolic pathways, participate in heat production, in upholding the equilibrium of reactive oxygen species, in Ca^{2+} metabolism, and in the control of apoptotic procedures of the cells [12].

1.1.1 The structures of mitochondria

Mitochondria have two membranes, an inner and an outer membrane. The outer membrane is between the extramitochondrial space (cytoplasm) and intermembrane space, the inner membrane is between the intermembrane space and mitochondrial matrix. The outer membrane is permeable for small molecules (ca. 6 kDa). This transport takes place across the porins, which are channels in the outer membrane. Because of the great permeability of the outer membrane, the concentrations of the small molecules and ions are similar in the cytoplasm and intermembrane space. Permeability of the inner membrane is smaller than of the outer membrane, because in this the location controlled transport of molecules takes place. In addition the surface of the inner membrane is bigger than the outer, because it has wrinkles (cristae). In the matrix one can find the enzymes of the citric acid cycle, which provide the energy production, the main function of mitochondria [13].

Structure of Mitochondria.

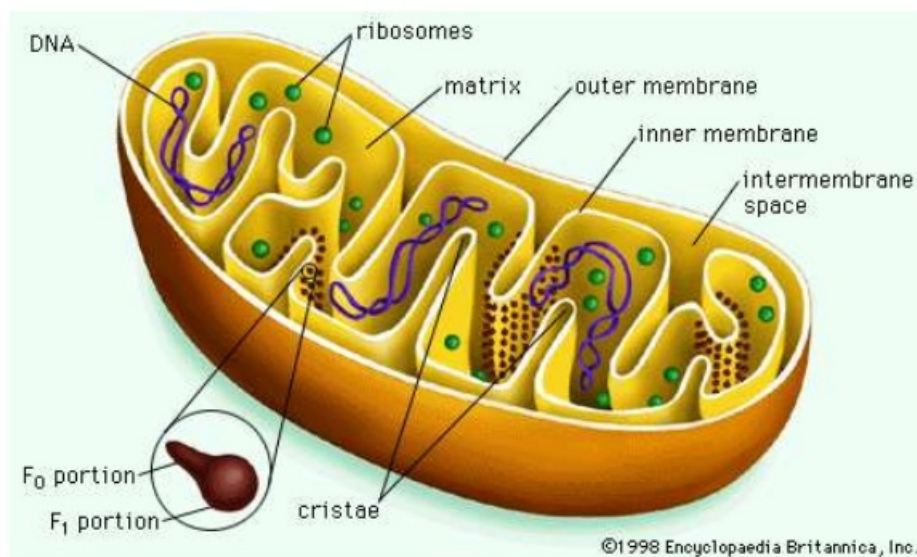


Figure 1.1: Structures of Mitochondria [14]

1.1.2 Bioenergetics

Mitochondria are able to achieve aerobic oxidation of molecules, these organelles take great part of energy generation in the cell. For example in the glucose metabolism, a glucose molecule is metabolized with oxygen. During this process mitochondrion produces CO_2 , H_2O and energy, which is stored in ATP molecules [15]. This process has three parts. First is glycolysis, which is localized in the cytoplasm, during which glucose is metabolized to two pyruvate molecules [16]. After that pyruvates are oxidised and connect to a transfer molecule (coenzyme A). This connected molecule is able to be used by citric acid cycle. Second part of metabolism of glucose (and a lot of molecules, proteins, fats and nucleic acids) takes place in the matrix of the mitochondrion with the help of a complex enzyme system, which is called citric acid cycle. This enzyme system can receive the intermediates which are produced by catabolic processes. During the reactions of citric acid cycle intermediates are metabolized or/and can be transformed into each other and energy, which in turn produces reduced equivalents (NADH , FADH_2) [16]. Third part of the metabolism is terminal oxidation, which contains the oxi-

dation of the reduced equivalents. When the reduced equivalents are oxidised the protons are transported out from the matrix, and build the electrochemical gradient. After that the protons transport back to the matrix through the ATP-synthase and with the gained energy ATP molecules are produced.

Citric acid cycle

Every mitochondrion contains a complex enzyme system, which is able to transform intermediates from the degradation of macromolecules (carbohydrates, proteins, fats, nucleic acids) into each other or able to catabolize that in terminal oxidation, and produce energy (ATP) [17]. Some of these reactions take part in production of reduced equivalents, which transport electrons to the complexes of respiratory chain, where these molecules are oxidized and energy is conserved in ATP molecules. Intermediates of the citric acid cycle are able to leave the cycle via transporters and able to become precursors of anabolic procedures. The cycle contains reversible reactions where the change in the Gibbs free energy is small, and irreversible reactions where the change is large. During these procedures energy is conserved in reduced equivalents, which can become oxidized in the terminal oxidation and store energy in form of ATP for the functions of cell.

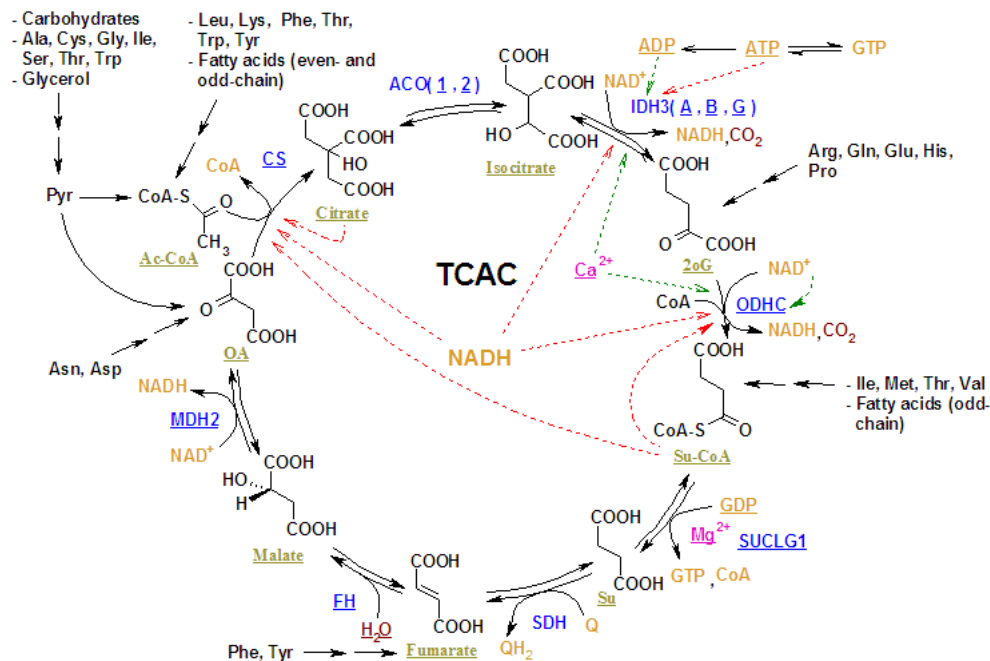


Figure 1.2: Citric acid cycle [18]

Heat production

In the brown adipocytes the mitochondria take great part of heat regulation of the body. With their uncoupling-proteins (UCP) mitochondria can achieve heat production from proton electrochemical gradient instead of ATP production via ATP-synthase [19].

1.1.3 Other functions of mitochondria

ROS homeostasis

The main source of reactive oxygen species (ROS) is the NADPH oxydase, which is activated in phagocytes during the inflammation mechanism. ROS which are produced by mitochondria take part in the regulation of the cell [20], modulate immunology processes [21, 22], take part in apoptosis [23], cell differentiation [24], autophagy [25], regulation of cellular [26, 27], and tissue inflammation [28]. With these functions mitochondria is able to modify cell regulation in ways which depend on the actual metabolic status of the mitochondria and cell.

Ca²⁺ homeostasis

In the cell a lot of function is regulated via amount of intracellular Ca²⁺. Mitochondria are able to get or release Ca²⁺, are able to buffer the amount of Ca²⁺. Intramitochondrial Ca²⁺ can activate a lot of enzymes included the rate limit enzymes in the citric acid cycle (pyruvate dehydrogenase, isocitrate dehydrogenase, α -ketoglutarate dehydrogenase). Because of this mitochondria are able to regulate the metabolism of the cell [29].

Apoptosis

Apoptosis is the programmed cell death, which is an important mechanism in regulating bodily functions. During this process the body can eliminate injured, infected or aged cells. Apoptosis is initialized by two different pathways. Extrinsic pathway is activated if the death receptors bind death ligands to the surface of the cell. This interaction initiates the caspase (cysteine aspartate protease) chain reaction, which leads to apoptosis. Mitochondria take part of the intrinsic pathway of the apoptosis. BCL2 proteins are anti-apoptotic factors, which are localized in the mitochondria. In case of lack of BLC2 proteins, cytochrome c is relieved from mitochondria, which activates the caspase system, and causes apoptosis [30, 31].

1.2 Previous measurements and results related to our work

In the Neurobiochemistry working group (Institute of Medical Biochemistry, Semmelweis University), we have taken enzyme kinetic measurements of several mitochondrial enzymes, and we have determined and analyzed interactions between enzymes and substrates. We have measured reactive oxygen species productions and the mitochondrial O_2 consumptions with different respiratory substrates [2, 3, 4, 5]. The need naturally arises for further analysis of the process. Therefore, a suitable quantitative (mathematical) model is constructed which describes the temporal changes in the quantities of citric acid cycle's molecules. The modeling goal in this research phase is to describe qualitatively the increased/decreased operation of the catalyzing enzymes as well as the modified operation of the intermediate molecules. In next phase we would like to be able to predict whether mitochondria have the ability to adapt to this new metabolic state by activating/inhibiting other metabolic pathways or the metabolism will become pathological.

In previous studies similar models [6, 7, 8, 9, 10] were constructed, it falls under our goals to promote further development compared to the known models, to combine them with each other and to expand them in other aspects.

In the first article [6] measurements of mitochondria from striated and cardiac muscle are used for the determination and validation of metabolic and electrophysiologic parameters. This model uses rapidly evolving equilibrium state which is determined by kinetic and thermodynamical equilibrium. The model contains many parameters that should be determined appropriately. The parameter values cover a wide range and depend on the amount of substrate concentration, measurements methods, and other physical and physiological parameters. The model can predict the individual factors effects (NADH, ATP, metabolic fluxes) in the regulation of citric acid cycle function and projects the effect of pH and membrane potential changes during ATP synthesis.

The second article [7] describes the simplified model of citric acid cycle, electron transport chain and ATP synthesis and their relations to each other. In this model the number of reaction equations is reduced significantly and usually uses irreversible instead of reversible reactions. Due to this mathematical analysis becomes easier. Describing the reactions model, the authors use differential equations which can show changes in concentrations during the simulated time. Additionally, this model takes in account the effect of membrane potential during ATP synthesis.

In the third article [8] examination of the process of the citric acid cycle, electron transport chain and ATP synthesis is described. Reactions of the citric acid cycle are described with eight differential equations. Reaction rates are described with Michaelis Menten reaction kinetic equation. Dynamic modeling of electron transport chain and ATP synthesis are described by the difference of the pH between two sides of the membrane. After that the model examines the ATP synthesis the function of O_2 concentration and intramitochondrial pH.

The fourth model [9] extends the earlier model [8]) with two transporter systems, which are the malate-aspartate and citrate-pyruvate shuttles. These transporter systems transport the NADH molecules between the intermembrane space and mitochondrial matrix which regenerates the cytoplasmic NAD^+ pool. Additionally, because of the changing mechanism these shuttle systems can keep the equilibrium of the different number of carbon chains in the mitochondrial matrix. This model presents the set of the equilibrium states of intermediates and visualizes the changes in time of intermediates in phase-plots.

In the fifth article [10] the author extends her previous model with production and elimination of reactive oxygen species, and their association with citric-acid cycle and electron transport chain. After that inhibitors and their effects of the metabolites are examined.

1.3 Further goals

The most important goal of the modeling is to describe the functions of mitochondria in healthy and pathologic states with a dynamic system. We would like to test this model under different pathological conditions and compare our results with the physiological measurements. If the model is accepted, it could be used in simulations of modified metabolic status in various enzyme defects by modifying the parameters of examined enzymes. With this model we plan to predict the altered metabolic state of the pathologic status, and to get a forecast about the mitochondrion, which could either adapt to the modified metabolic status or lead to pathological functions, and death of the mitochondrion.

Chapter 2

Kinetic modeling

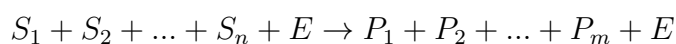
2.1 Enzymes of the citric cycle

Enzymes are macromolecules which catalyze biochemical reactions via reducing the necessary activation energy. They increase the probability of reactions, but the energy change in the reaction is not affected. At the end of the reaction, we will get back the enzymes unchanged. Certain enzyme functions can be greatly influenced by various environmental effects (temperature, pH, ion concentration).

Enzymes are typically proteins, but there are RNA enzymes (ribozyme) as well. Proteins can be simple types of enzymes, which contain only parts of proteins (subunits) or complex if they contain non-protein components. If these components are covalently bound to the enzyme, we are talking about prosthetic groups, while if only loosely bound molecules are concerned, we are talking about coenzymes.

Other grouping options are based on the reaction-type, which is catalyzed by the enzyme. Classification of enzymes (EC number) in the register marks the type of the reaction. Thus we can discriminate between oxidoreductases (1, eg: malate dehydrogenase (1.1.1.37)), transferases (2, eg: aspartate transaminase (2.6.1.1)), hydrolases (3), lyases (4, eg: citrate synthase (4.1.3.7)), isomerases (5), ligases (6, eg: succinyl-CoA ligase (6.2.1.4)) [32].

During a reaction an enzyme (E) produces a product (P) from one (or more) of the starting molecules (substrate, S). The order of a reaction means a number that shows on how many substrate concentrations the reaction rate depends. In certain cases all the concentration of the substrates can influence the reaction. The general description of a reaction, if we have n substrates:

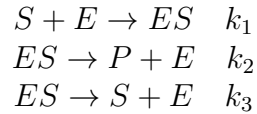


2.2 Michaelis Menten kinetics

In the simplest case one substrate turns into one product and at the end of the reaction, the enzyme is returned unchanged. The reaction can be regarded as two reaction steps. In the first step, which is a reversible process, the enzyme binds to the substrate and forms an enzyme-substrate complex (ES). After that ES dissociates to S, E is thus again generated. The other case is when this happens in catalysis, the substrate becomes product. In the model it is assumed that during the first step instantaneously happens in the complex and the concentration is kept constant for a long time (steady state). Concentration is difficult to measure, it is determined by the other parameters. Furthermore, it is assumed that the decrease of the substrate is negligible, and that the resulting product is not converted back [33, 34].



Then we examine the three elementary reactions and the associated rate constants:



[ES] volume is considered constant (steady state), so the change is 0. This is true, because the change in the amount of ES complex is negligible compared to the loss of substrate and product formation. This constant value of the rate constant (k_1 , k_2 , k_3) and [E] and [S] values define, what can be calculated as follows:

$$\frac{d}{dt}[ES] = k_1[S][E] - k_2[ES] - k_3[ES] = 0 \quad (2.1)$$

After that rearranging the equation (2.2), we know that the enzyme is present in free form or in substrate binds, and this is the total amount of enzyme ($[E_t]$), (2.3):

$$k_1[S][E] = k_2[ES] + k_3[ES] \quad (2.2)$$

$$[E] = [E_t] - [ES] \quad (2.3)$$

[E] place in equation 2.2 is written to equation 2.3, we can express [ES] as follows:

$$[ES] = \frac{k_1[S][E_t]}{k_1[S] + k_2 + k_3} = \frac{[S][E_t]}{[S] + \frac{k_2+k_3}{k_1}} \quad (2.4)$$

After that we will introduce the Michaelis Menten constant (K_M [35]). This value represents the substrate concentration at which the enzyme reaction rate is the half of the maximum rate. (We will show this straightway.) Then substituting K_M to equation 2.4:

$$K_M = \frac{k_2 + k_3}{k_1} \quad (2.5)$$

$$[ES] = \frac{[S][E_t]}{[S] + K_M} \quad (2.6)$$

[ES] value is determined (2.6). The product formation rate (V) only depends on [ES] value and k_2 reaction rate (2.7). We know that is maximal, for all the enzyme binds to the substrate (2.8):

$$V = k_2[ES] = \frac{k_2[S][E_t]}{[S] + K_M} \quad (2.7)$$

$$[ES] = [E_t] \Rightarrow V_{max} = k_2[E_t] \quad (2.8)$$

After that we substitute equation 2.8 to equation 2.7:

$$V = \frac{V_{max}[S]}{[S] + K_M} \quad (2.9)$$

We obtained reaction rate of the given substrate concentration (2.9). If we substitute the Michaelis Menten constant (K_M) to the equation 2.9, we can see this concentration is really the half of the maximum reaction rate:

$$[S] = K_M \Rightarrow V = \frac{V_{max}[S]}{[S] + K_M} = \frac{V_{max}K_M}{K_M + K_M} = \frac{V_{max}}{2} \quad (2.10)$$

2.3 Modeling the reactions

2.3.1 Single component reactions

Reaction rate of single component reactions depends on substrate concentration, enzyme affinity to the substrate and maximum reaction rate of the enzyme. This connection is described with the Michaelis Menten equation, which was presented in the previous section. $V_{max}(E)$ is a parameter which depends on the enzyme, $[S]$ is the concentration of the substrate, $K_M(S,E)$ is the Michaelis Menten constant which depends on enzyme and substrate. $V_{S \rightarrow P}$ is the reaction rate of the elemental reaction.

$$V_{S \rightarrow P} = \frac{V_{max}(E)[S]}{[S] + K_M(S,E)}$$

2.3.2 Description of reversible reactions

We can model a reversible reaction as two elementary reaction steps which occur in different directions.

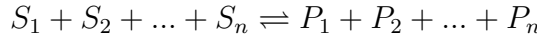
$$V_{S_1 \rightarrow S_2} = \frac{V_{max}(E)[S_1]}{[S_1] + K_M(S_1,E)}$$

$$V_{S_2 \rightarrow S_1} = \frac{V_{max}(E)[S_2]}{[S_2] + K_M(S_2,E)}$$

$$\begin{aligned} V_{S_1 \rightleftharpoons S_2} &= \frac{V_{max}(E)[S_1]}{[S_1] + K_M(S_1,E)} - \frac{V_{max}(E)[S_2]}{[S_2] + K_M(S_2,E)} = \\ &= V_{max}(E) \cdot \left(\frac{[S_1]}{[S_1] + K_M(S_1,E)} - \frac{[S_2]}{[S_2] + K_M(S_2,E)} \right) \end{aligned}$$

2.3.3 Multicomponent reactions

In multicomponent reactions reaction rates depend on all of the substrate concentrations, and because of this reaction rates can be computed, if we multiply the single reaction rates of the substrates.



$$V_{S_1, S_2, \dots, S_n} = V_{max}(E) \cdot \left(\frac{[S_1]}{[S_1] + K_M(S_1, E)} \right) \cdot \left(\frac{[S_2]}{[S_2] + K_M(S_2, E)} \right) \cdot \dots \cdot \left(\frac{[S_n]}{[S_n] + K_M(S_n, E)} \right)$$

$$V_{P_1, P_2, \dots, P_n} = V_{max}(E) \cdot \left(\frac{[P_1]}{[P_1] + K_M(P_1, E)} \right) \cdot \left(\frac{[P_2]}{[P_2] + K_M(P_2, E)} \right) \cdot \dots \cdot \left(\frac{[P_n]}{[P_n] + K_M(P_n, E)} \right)$$

$$\begin{aligned} V_{S \rightleftharpoons P} &= V_{S_1, S_2, \dots, S_n} - V_{P_1, P_2, \dots, P_n} = \\ &= V_{max}(E) \cdot \left(\left(\frac{[S_1]}{[S_1] + K_M(S_1, E)} \right) \cdot \left(\frac{[S_2]}{[S_2] + K_M(S_2, E)} \right) \cdot \dots \cdot \left(\frac{[S_n]}{[S_n] + K_M(S_n, E)} \right) - \right. \\ &\quad \left. - \left(\frac{[P_1]}{[P_1] + K_M(P_1, E)} \right) \cdot \left(\frac{[P_2]}{[P_2] + K_M(P_2, E)} \right) \cdot \dots \cdot \left(\frac{[P_n]}{[P_n] + K_M(P_n, E)} \right) \right) \end{aligned}$$

Stoichiometric matrix

In the multicomponent reactions we use a stoichiometric matrix ($S_{n \times k}$), which contains the stoichiometric coefficients in its columns for every reactions (n pieces of reactions), and in its rows it contains the substrates (k pieces of substrates). This matrix is multiplied by the reaction rate vector (V_n), which is calculated with Michaelis Menten kinetics every reactions. Result of this multiplication gives the changes of substance in time.

$$\frac{d}{dt}X = S_{n \times k} \cdot V_n$$

The reversible reactions are described in the stoichiometric matrix with two columns with negative values, because the two reactions have different reaction rates. In the reversible reactions (where i and i+1 are indexes of the two elemental reactions) the change of concentration of the initial substrate can be described with this equation.

Reaction rate vector

In our model reactions are modeled with extended Michaelis Menten kinetic model, which means that the reaction rates depend on all of the initial substrate concentrations. In this case Michaelis Menten kinetics can be used to determine reaction rate vector (V). V_j is the rate of j^{th} reaction, $[X_k]$ is the concentration of k^{th} substrate, $K_{M_{k,j}}$ Michaelis Menten constant of k^{th} substrate of the j^{th} enzyme and $V_{max,j}$ is the maximum rate of the j^{th} enzyme. If the k^{th} substrate does not take a role of the j^{th} reaction, then we use $K_{M_{k,j}} = 0$.

$$V_1 = V_{max,1} \cdot \left(\frac{[X_1]}{[X_1] + K_{M_{1,1}}} \cdot \frac{[X_2]}{[X_2] + K_{M_{2,1}}} \cdot \dots \cdot \frac{[X_k]}{[X_k] + K_{M_{k,1}}} \cdot \dots \cdot \frac{[X_n]}{[X_n] + K_{M_{n,1}}} \right)$$

$$V_2 = V_{max,2} \cdot \left(\frac{[X_1]}{[X_1] + K_{M_{1,2}}} \cdot \frac{[X_2]}{[X_2] + K_{M_{2,2}}} \cdot \dots \cdot \frac{[X_k]}{[X_k] + K_{M_{k,2}}} \cdot \dots \cdot \frac{[X_n]}{[X_n] + K_{M_{n,2}}} \right)$$

$$\vdots$$

$$V_j = V_{max,j} \cdot \left(\frac{[X_1]}{[X_1] + K_{M_{1,j}}} \cdot \frac{[X_2]}{[X_2] + K_{M_{2,j}}} \cdot \dots \cdot \frac{[X_k]}{[X_k] + K_{M_{k,j}}} \cdot \dots \cdot \frac{[X_n]}{[X_n] + K_{M_{n,j}}} \right)$$

$$\vdots$$

$$V_n = V_{max,n} \cdot \left(\frac{[X_1]}{[X_1] + K_{M_{1,n}}} \cdot \frac{[X_2]}{[X_2] + K_{M_{2,n}}} \cdot \dots \cdot \frac{[X_k]}{[X_k] + K_{M_{k,n}}} \cdot \dots \cdot \frac{[X_n]}{[X_n] + K_{M_{n,n}}} \right)$$

Chapter 3

Elements of the model

Mitochondrial metabolism is not only different in the various species, but in different organs also works differently. The amount and the activity of each enzyme depends on the high concentration of substrates, which usually increases the activity and usually the product is able to inhibit enzymes (negative feedback). Therefore, when creating the model we tried to search for the parameters of mitochondrial enzymes under similar conditions. If we don't find a parameter, we use "not found" in its place, but in the future there will be more accurate measurement data available, it will be possible to further calibrate the model.

3.1 Enzymes of the citric acid cycle, and their major parameters

In this work we modeled the reactions of citric acid cycle, electron transport chain, malate-aspartate and citrate-pyruvate shuttles. The following list contains the reactions and their catalyzing enzymes/transporters involved in our model (Number of reaction, name of enzyme, abbreviation in the model, Enzyme Commission (EC) number of enzyme).

R1: Citrate synthase (CS, 4.1.3.7)

Catalysed reaction:

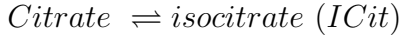
Acetyl-CoA (*AcCoA*) + *oxaloacetate* (*OA*) → *citrate* (*Cit*)

Parameters [36]:

$V_{max} = 1.88 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$; $K_{M,OA} = 5.9 \mu\text{M}$; $K_{M,AcCoA} = 5 \mu\text{M}$;

R2: Aconitase (ACON, 4.2.1.3)

Catalysed reaction:



Parameters [37]:

$$V_{max} = 3.4 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}; K_{M,Cit} = 480 \mu\text{M}; K_{M,ICit} = 120 \mu\text{M};$$

R3: Isocitrate dehydrogenase (IDH, 1.1.1.41)

Catalysed reaction:

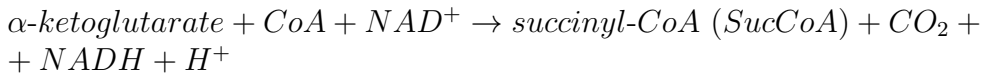


Parameters [38]:

$$V_{max} = 21.7 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}; K_{M,ICit} = 2200 \mu\text{M}; K_{M,NAD^+} = 73 \mu\text{M};$$

R4: α -ketoglutarate dehydrogenase (α KGDH, 1.2.4.2)

Catalysed reaction:



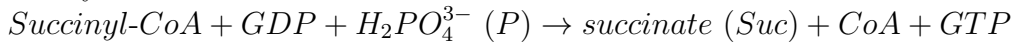
Parameters [39, 40]:

$$V_{max} = 0.2 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}; K_{M,\alpha\text{KG}} = 117 \mu\text{M}; K_{M,NAD^+} = \text{not found};$$

$$K_{M,CoA} = \text{not found};$$

R5: Succinyl-coenzyme A syntase (SCS, 6.2.1.4)

Catalysed reaction:



Parameters [41]:

$$V_{max} = 22.1 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}; K_{M,SucCoA} = 86 \mu\text{M}; K_{M,GDP} = 7 \mu\text{M};$$

$$K_{M,P} = 2260 \mu\text{M};$$

R6: Succinate dehydrogenase (SDH, 1.3.5.1)

Catalysed reaction:

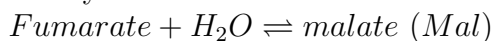


Parameters [42]:

$$V_{max} = 41 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}; K_{M,Suc} = 20 \mu\text{M};$$

R7: Fumarase (FH, 4.2.1.2)

Catalysed reaction:



Parameters [43]:

$$V_{\max} = 450 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}; K_{M,\text{Fum}} = 5 \mu\text{M}; K_{M,\text{Mal}} = 25 \mu\text{M};$$

R8: Malate dehydrogenase (MDH-m, 1.1.1.37), mitochondrial

Catalysed reaction:



Parameters [44]:

$$V_{\max} = 110 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}; K_{M,\text{Mal}} = 300 \mu\text{M}; K_{M,\text{OA}} = 34.8 \mu\text{M};$$

$$K_{M,\text{NAD}^+} = 140 \mu\text{M}; K_{M,\text{NADH}} = 14.5 \mu\text{M};$$

3.2 Coenzymes of citric cycle, and their role in the metabolism

Coenzyme A

Coenzyme A (CoA) is a carrier molecule, which can carry functional groups (eg. acetyl, succinyl, malonyl or propionyl group) during metabolism.

Acetyl-CoA

Acetyl-coenzyme A is a molecule, that is formed from pyruvic acid or in the oxidation of fatty acids. It delivers acetyl groups and with oxaloacetate turns into citrate, which is the initial molecule in the citric acid cycle.

NAD⁺

The nicotinamide adenine dinucleotide is a participant of the mitochondrial electron transport, this molecule can carry two electrons to the complex I in the respiratory chain. After that the electrons are in the electron transport chain, thus provide energy for the functioning of ATP synthase which is responsible for the $\text{ADP} \rightarrow \text{ATP}$ transformation.

FAD

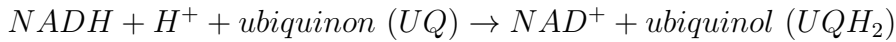
Flavin adenine dinucleotide is the prosthetic group of succinate dehydrogenase, which can carry two electrons to the electron transport chain. Energy of this electrons goes into ATP synthesis.

3.3 Enzymes of electron transport chain, and their major parameters

In citric acid cycle operation during a certain reaction NAD^+ and FAD molecules are reduced. After that in the electron transport chain NADH on the complex I, FADH_2 on the complex II are able to oxidize. The reactions occurring during the complex-I 4 H^+ are flowing out of the space between the two membranes of mitochondrion, thereby creating an electrochemical gradient between the two sides of the membrane. During the complex-II reaction H^+ is not pumped between two mitochondrial membranes. During the oxidation electrons of H-s move from complex I and II to the complex III, which pumps further 4 H^+ from the matrix to the intermembrane space. After that the electrons to pass through to complex IV, which delivers further 2 H^+ out from the matrix of mitochondrion. H^+ can return to the matrix through ATP synthase, this process moves in the electrochemical gradient's direction, during this reaction the energy is conserved by $\text{ADP} \rightarrow \text{ATP}$ transformation. The returned H^+ , electrons which traveled along the transport chain and O_2 are combined to water.

R9: NADH:ubiquinon oxidoreductase Complex-I (C1, 1.6.5.11)

Catalysed reaction:

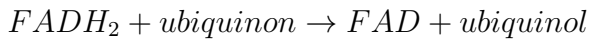


Parameters [45]:

$$V_{\max} = \text{not found}; K_{M,\text{NADH}} = 12 \mu\text{M}; K_{M,\text{UQ}} = \text{not found};$$

R10: Succinate dehydrogenase, Complex-II (C2, 1.3.5.1)

Catalysed reaction:

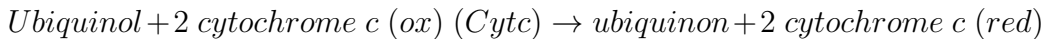


Parameters [42]:

$$V_{\max} = 41 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}; K_{M,\text{FADH}_2} = \text{not found}; K_{M,\text{UQ}} = 0.5 \mu\text{M};$$

R11: Quinol:cytochrome-c reductase, Complex-III (C3, 1.10.2.2)

Catalysed reaction:

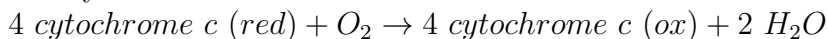


Parameters [46, 47]:

$$V_{\max} = 65 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}; K_{M,\text{UQH}_2} = 1 \mu\text{M}; K_{M,\text{Cyt c(ox)}} = 9 \mu\text{M};$$

R12: Cytochrome oxidase, Complex-IV (C4, 1.9.3.1)

Catalysed reaction:



Parameters [48, 49, 50]:

$$V_{max} = 0.51 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}; K_{M,Cytc(\text{red})} = 37 \mu\text{M}; K_{M,O_2} = 1 \mu\text{M};$$

3.4 Transporters of mitochondrion

Inner membrane of mitochondrion is impermeable to most molecules. Because of this the different substances are able to move across the inner membrane only via transporters, between the intermembrane space and matrix. These transporters are uniporters (transports one substance), symporters (transports more substances in the same direction) or antiporters (transports more substances in different directions). SLC transporter family contains many transporters which take part of transportation of the substrates of citric acid cycle [51].

In the model functions of transporter are described with Michaelis Menten kinetics similar to the enzymes. The model contains one uniporter (pyruvate transporter, PT), which can transport pyruvate in the direction of the concentration gradient, and four antiporters (citrate transporter (CTP), dicarboxylate carrier (DIC), α -ketoglutarate transporter (α KGT), aspartate glutamate carrier (AGC)), which transport two molecules to different directions with 1:1 stoichiometry. Because of this if a substance transports across the membrane an other substance moves back. During the transports ions which have been changed between the two sides of the inner membrane have similar charge, because of this the difference of the concentrations between the two sides of the membrane causes a driving force. In this model substrate concentrations change in the matrix because of enzymes and transports. Transport of a substrate is calculated from the difference of inward and outward fluxes. In parallel the other substance which participates in the transport moves to a different direction. Enzymes and transporters take part in functional units, which are called shuttles. In this model we use malate-aspartate and citrate-pyruvate shuttles.

3.5 Malate-aspartate shuttle

This shuttle's function is the NADH transport from the cytoplasm to the matrix and to regenerate cytoplasmic NAD^+ pool. This process uses four enzymes and two transporters: malate dehydrogenase (mitochondrial (MDH-m) and cytoplasmic (MDH-c) isozyme), aspartate aminotransferase/glutamate oxaloacetate transaminase (ASAT/GOT, mitochondrial (ASAT-m) and cytoplasmic (ASAT-c) isozyme), α -ketoglutarate transporter (α KGT) and aspartate glutamate carrier (AGC).

3.5.1 Reactions of the shuttle

1. Oxaloacetate + $NADH + H^+ \rightarrow$ malate + NAD^+ (cytoplasm, MDH-c)
2. Cytoplasmic malate and mitochondrial α KG exchange (α KGT)
3. Malate + $NAD^+ \rightarrow$ oxaloacetate + $NADH + H^+$ (matrix, MDH-m)
4. Oxaloacetate + glutamate $\rightarrow$ aspartate + α KG (matrix, ASAT-m)
5. Mitochondrial aspartate and cytoplasmic glutamate exchange (AGC)
6. Aspartate + α KG $\rightarrow$ oxaloacetate + glutamate (cytoplasm, ASAT-c)

3.5.2 Enzymes of malate-aspartate shuttle

R13: Malate dehydrogenase (MDH-c, 1.1.1.37), cytoplasmic

Catalysed reaction:



Parameters [48, 49, 50]:

$$V_{max} = 110 \frac{\mu mol}{min \cdot mg}; K_{M,Mal} = 300 \mu M; K_{M,OA} = 8.3 \mu M;$$

$$K_{M,NAD^+} = 140 \mu M; K_{M,NADH} = 20.5 \mu M;$$

Malate dehydrogenase (MDH-m, 1.1.1.37), mitochondrial

Further information in section "Enzymes of citric acid cycle, and their major parameters"

R14: Aspartate aminotransferase (ASAT-m, 2.6.1.1), mitochondrial

Catalysed reaction:

Oxaloacetate + glutamate (Glu) $\rightleftharpoons$ aspartate (Asp) + α -ketoglutarate

Parameters [52]:

$V_{max} = 22.37 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$; $K_{M,OA}$ = not found; $K_{M,\alpha KG} = 1750 \mu\text{M}$;
 $K_{M,Asp} = 750 \mu\text{M}$; $K_{M,Glu}$ = not found;

R15: Aspartate aminotransferase (ASAT-c, 2.6.1.1), cytoplasmic

Catalysed reaction:

Oxaloacetate + glutamate $\rightleftharpoons$ aspartate + α -ketoglutarate

Parameters [52]:

$V_{max} = 48.45 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$; $K_{M,OA}$ = not found; $K_{M,\alpha KG} = 1250 \mu\text{M}$;
 $K_{M,Asp} = 350 \mu\text{M}$; $K_{M,Glu}$ = not found;

3.5.3 Transporters of malate-aspartate shuttle**R16: α -ketoglutarate transporter (α KGT)**

Synonym: α -ketoglutarate/malate carrier

Gene (locus): SLC25A11 (17p13.3), SLC20A4

Substrates: α -ketoglutarate, malate

Transport: α -ketoglutarate $\rightleftharpoons$ malate

Localisation: heart, striated muscle, liver, kidney, brain, pancreas

Parameters [53]:

Malate: $V_{max} = 10000 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$; $K_{M,in} = 710 \mu\text{M}$; $K_{M,out} = 1360 \mu\text{M}$;
 α -ketoglutarate: $V_{max} = 9500 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$; $K_{M,in} = 170 \mu\text{M}$; $K_{M,out} = 310 \mu\text{M}$;

R17: Aspartate glutamate carrier 1 (AGC1)

Synonym: Aralar1

Gene (locus): SLC25A12 (2q24)

Substrates: aspartate, α -ketoglutarate

Transport: aspartate $\rightleftharpoons$ α -ketoglutarate + H^+

Localisation: brain, heart, striated muscle, pancreas, kidney, lung

Parameters [53, 54]:

Aspartate: $V_{max} = 49 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$; $K_{M,in} = 216 \mu\text{M}$; $K_{M,out} = 52 \mu\text{M}$;
 Glutamate: $V_{max} = 52 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$; $K_{M,in} = 52 \mu\text{M}$; $K_{M,out} = 216 \mu\text{M}$;

R18: Aspartate glutamate carrier 2 (AGC2)

Synonym: Citrin, CTLN2, Aralar2

Gene (locus): SLC25A13 (7q21.3)

Substrates: aspartate, α -ketoglutarate

Transport: aspartate $\rightleftharpoons$ α -ketoglutarate + H⁺

Localisation: brain, heart, striated muscle, pancreas, kidney, liver

Parameters [53, 54]:

Aspartate: $V_{max} = 185 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$; $K_{M,in} = 205 \mu\text{M}$; $K_{M,out} = 50 \mu\text{M}$;

Glutamate: $V_{max} = 172 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$; $K_{M,in} = 50 \mu\text{M}$; $K_{M,out} = 205 \mu\text{M}$;

Disease association: Type II deficiency causes type II citrullinaemia (CTLN2) [55], and neonatal intrahepatic cholestasis (NICCD) [56]

3.6 Citrate-pyruvate shuttle

This shuttle function is responsible for the acetyl-CoA transport from the mitochondrial matrix to the cytoplasm for fatty acid synthesis, because the inner membrane is impermeable to acetyl-CoA. This process uses seven enzymes and three transporters: ATP citrate synthase (ACS), malate dehydrogenase (MDH-m and MDH-c), malic enzyme (ME), pyruvate dehydrogenase (PDH), pyruvate carboxylase (PC), citrate synthase (CS), citrate transporter (CTP), dicarboxylate transporter (DIC) pyruvate transporter (PT).

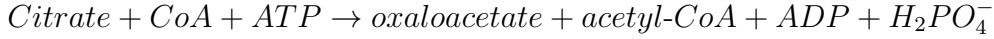
3.6.1 Reactions of the shuttle

1. Mitochondrial citrate and cytoplasmic malate exchange (CTP)
2. Mitochondrial malate and cytoplasmic HPO_4^{2-} exchange (CTP)
3. $\text{Malate} + \text{NAD}^+ \rightarrow \text{oxaloacetate} + \text{NADH} + \text{H}^+$ (matrix, MDH-m)
4. $\text{Citrate} + \text{CoA-SH} + \text{ATP} \rightarrow \text{oxaloacetate} + \text{acetyl-CoA} + \text{ADP} + \text{HPO}_4^{2-}$ (cytoplasm, ACS)
5. $\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \rightarrow \text{malate} + \text{NAD}^+$ (cytoplasm, MDH-c)
6. $\text{Malate} + \text{NADP}^+ \rightarrow \text{pyruvate} + \text{NADPH} + \text{H}^+ + \text{CO}_2$ (cytoplasm, ME)
7. Cytoplasmic pyruvate to the matrix (PT)
8. $\text{Pyruvate} + \text{CoA-SH} + \text{NAD}^+ \rightarrow \text{acetyl-CoA} + \text{NADH} + \text{H}^+ + \text{CO}_2$ (matrix, PDH)
9. $\text{Pyruvate} + \text{ATP} + \text{CO}_2 \rightarrow \text{oxaloacetate} + \text{ADP} + \text{HPO}_4^{2-}$ (matrix, PC)
10. $\text{Oxaloacetate} + \text{acetyl-CoA} \rightarrow \text{citrate}$ (matrix, CS)

3.6.2 Enzymes of citrate-pyruvate shuttle

R19: ATP citrate syntase (ACS, 2.3.3.8)

Catalysed reaction:



Parameters [57, 58]:

$$V_{\max} = 13.6 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}; K_{M,\text{Cit}} = 110 \mu\text{M}; K_{M,\text{ATP}} = 200 \mu\text{M}; \\ K_{M,\text{CoA}} = 4.2 \mu\text{M};$$

Malate dehydrogenase (MDH-m, 1.1.1.37), mitochondrial

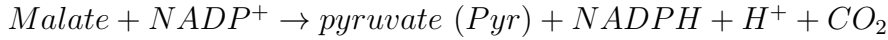
Further information in section "Enzymes of citric acid cycle, and their major parameters"

Malate dehydrogenase (MDH-c, 1.1.1.37), cytoplasmic

Further information in section "Malate-aspartate shuttle"

R20: Malic enzyme (ME, 1.1.1.40)

Catalysed reaction:



Parameters [59]:

$$V_{\max} = 56.35 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}; K_{M,\text{Mal}} = 120 \mu\text{M}; K_{M,\text{NADP}^+} = 9.2 \mu\text{M};$$

R21: Pyruvate dehydrogenase (PDH, 1.2.4.1)

Catalysed reaction:

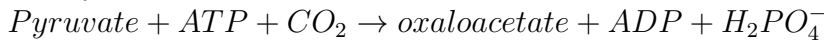


Parameters [60]:

$$V_{\max} = 5.7 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}; K_{M,\text{Pyr}} = 0.2 \mu\text{M}; K_{M,\text{NAD}^+} = 33 \mu\text{M}; K_{M,\text{CoA}} = 4 \mu\text{M};$$

R22: Pyruvate carboxylase (PC, 6.4.1.1)

Catalysed reaction:



Parameters [61]:

$$V_{\max} = 20 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}; K_{M,\text{Pyr}} = 220 \mu\text{M}; K_{M,\text{ATP}} = 220 \mu\text{M}; \\ K_{M,\text{CO}_2} = 3200 \mu\text{M};$$

Citrate synthase (CS, 4.1.3.7)

Further information in section "Enzymes of citric acid cycle, and their major parameters"

3.6.3 Transporters of citrate-pyruvate shuttle**R23: Citrate transporter (CTP)**

Synonym: CIC (citrate carrier), tricarboxylate carrier

Gene (locus): SLC25A1 (22q11.21), SLC20A3

Substrates: citrate, isocitrate, malate, phosphoenolpyruvate (PEP)

Transport: $\text{citrate} + \text{H}^+ \rightleftharpoons \text{malate}$

Localisation: brain, lung, heart

Parameters [53]:

Citrate: $V_{max} = 10500 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$; $K_{M,in} = 27 \mu\text{M}$; $K_{M,out} = 32 \mu\text{M}$;

Malate: $V_{max} = 11500 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$; $K_{M,in} = 60 \mu\text{M}$; $K_{M,out} = 250 \mu\text{M}$;

R24: Dicarboxylate carrier (DIC)

Gene (locus): SLC25A10 (17q25.3)

Substrates: malate, phosphate, succinate, sulfate (SO_4^{2-}), tiosulfate ($\text{S}_2\text{O}_3^{2-}$)

Transport: $\text{H}_2\text{PO}_4^- \rightleftharpoons \text{malate}$

Localisation: liver, kidney, heart, brain, lung, pancreas

Parameters [53]:

Malate: $V_{max} = 6000 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$; $K_{M,in} = 920 \mu\text{M}$; $K_{M,out} = 490 \mu\text{M}$;

Phosphate: $V_{max} = 6000 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$; $K_{M,in} = 930 \mu\text{M}$; $K_{M,out} = 1410 \mu\text{M}$;

R25: Pyruvate transporter (PT) - ?

Synonym: Pyruvate carrier

Substrates: pyruvate

Transport: pyruvate via concentration gradient

Parameters [53]:

$V_{max} = 114 \frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$; $K_{M,in} = \text{not found}$; $K_{M,out} = 240 \mu\text{M}$;

3.7 Other transporters

Glutamate carrier 1-2 (GC1-2)

Gene (locus): SLC25A22 (11p15.5), SLC25A18 (22q11.2)

Substrates: Glutamate

Transport: Glutamate + H^+ to the matrix

Localisation: pancreas, brain, testis, kidney, heart, lung, liver (1), splen (1), intestine (1)

Disease association: Type I deficiency causes neonatal epileptic encephalopathy [62, 63, 64]

Uncoupling protein 1-5 (UCP1-5)

Synonym: Thermogenin (1), UCPH (2), Proton carrier (1-3), Brain mitochondrial carrier protein (BMCP1) (5)

Gene (locus): SLC25A7 (4q28-q31) (1), SLC25A8 (11q13) (2), SLC25A9 (11q13) (3), SLC25A27 (6p11.2-q12) (4), SLC25A14 (Xq24) (5)

Substrates: H^+ (1-3)

Transport: H^+ to the matrix (1-3)

Localisation: brown adipose tissue (1), lung (2, 3), kidney (2), splen (2), heart (2), striated muscle (3), brain (4, 5), testis (5)

Disease association: obesity (1, 2), type II diabetes (2, 3), familial hyperinsulinism (2)

Chapter 4

Quantitative model of the mitochondrial metabolism

4.1 Modeling goals

Our model simulates reactions of the citric acid cycle, electron transport chain, malate-aspartate and citrate-pyruvate shuttle. The modeling goals are the following:

1. To determine the concentrations of substrates that participate in the reactions in physiological conditions.
2. To predict the new equilibrium status after we modified initial conditions.
3. To model the changed dynamic of the system in case of specific diseases by using pathological parameters of enzymes and/or pathological initial conditions.

4.2 Structure of the model

Our model is an integrated structure, which is built from four different dynamic systems: citric acid cycle, electron transport chain, malate-aspartate shuttle and citrate-pyruvate shuttle. These systems have common (interfacing) molecules, which connect these modules to each other. For example reduced equivalents (NADH, FADH₂) are outputs of the citric acid cycle and inputs of the electron transport chain. Inputs of the shuttles are intermediate molecules of citric acid cycle. Malate, α -ketoglutarate and oxaloacetate take part in the malate-aspartate shuttle, citrate, malate and oxaloacetate take

part in the citrate-pyruvate shuttle. During the first step of the modeling we construct the individual modules, after that we build the connections. If we don't find a parameter, we use "not found = nf" in its place.

Conversions in every module was described by Michaelis Menten kinetics. Reversible reactions were described as two opposite elementary reactions. In these reactions we used different K_M values for different substrates. Changes of intermediates were described by differential equations. Every module needs input flux of some molecules, which keep up the steady state. When we connect the modules these inputs come from the other modules.

4.2.1 Module I: Citric acid cycle

In this module citric acid cycle (CAC) contains 8 enzymes. These enzymes catalyze 5 irreversible and 3 reversible reactions (Table 4.1). That generates 18 differential equations with 18 variables. This module needs 5 inputs (acetyl-CoA, NAD^+ , FAD, GDP, H_2PO_4^-) and it gives 4 outputs (NADH, FADH_2 , GTP, CO_2).

Stoichiometric matrix of the citric acid cycle

$$S_{CAC} = \begin{pmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 \\ 1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 1 \\ 0 & 0 & 0 & -1 & -1 & 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Enzyme and reaction	substrate	V_{max} ($\frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$)	K_M (μM)
Citrate synthase (CS) Acetyl-CoA + oxaloacetate $\rightarrow$ $\rightarrow$ citrate + CoA-SH	acetyl-CoA oxaloacetate	1.88	5 5.9
Aconitase (ACON) Citrate $\rightleftharpoons$ isocitrate	citrate isocitrate	3.4	480 120
Isocitrate dehydrogenase (IDH) Isocitrate + NAD ⁺ $\rightarrow$ $\rightarrow$ α -ketoglutarate + NADH + H ⁺ + CO ₂	isocitrate NAD ⁺	21.7	2200 73
α -ketoglutarate dehydrogenase (α KGDH) α -ketoglutarate + NAD ⁺ + CoA-SH $\rightarrow$ $\rightarrow$ succinyl-CoA + NADH + H ⁺ + CO ₂	α -ketoglutarate NAD ⁺	0.2	13 nf
Succinyl-CoA syntase (SCS) Succinyl-CoA + GDP + PO ₄ ³⁻ $\rightarrow$ $\rightarrow$ succinate + CoA-SH + GTP	succinyl-CoA GDP PO ₄ ³⁻	22.1	86 7 2260
Succinate dehydrogenase (SDH) Succinate + FAD $\rightarrow$ fumarate + FADH ₂	succinate FAD	41	20 nf
Fumarase (FH) Fumarate $\rightleftharpoons$ malate	fumarate malate	450	5 35
Malate dehydrogenase (MDH-m), mitochondrial Malate + NAD ⁺ $\rightleftharpoons$ $\rightleftharpoons$ oxaloacetate + NADH + H ⁺	malate oxaloacetate NAD ⁺ NADH	110	300 34.8 140 14.5

Table 4.1: Enzymes and reactions in the citric acid cycle

Reaction rate vector of the citric acid cycle

$$V_{CAC} = \begin{pmatrix} V_{CS} \cdot \frac{[OA]}{[OA] + K_{M(OA,CS)}} \cdot \frac{[AcCoA]}{[AcCoA] + K_{M(AcCoA,CS)}} \\ V_{ACON} \cdot \frac{[Cit]}{[Cit] + K_{M(Cit,ACON)}} \\ V_{ACON} \cdot \frac{[ICit]}{[ICit] + K_{M(ICit,ACON)}} \\ V_{IDH} \cdot \frac{[ICit]}{[ICit] + K_{M(ICit,IDH)}} \cdot \frac{[NAD^+]}{[NAD^+] + K_{M(NAD^+,IDH)}} \\ V_{\alpha KGDH} \cdot \frac{[\alpha KG]}{[\alpha KG] + K_{M(\alpha KG, \alpha KGDH)}} \cdot \frac{[NAD^+]}{[NAD^+] + K_{M(NAD^+, \alpha KGDH)}} \\ V_{SCS} \cdot \frac{[SucCoA]}{[SucCoA] + K_{M(SucCoA,SCS)}} \cdot \frac{[GDP]}{[GDP] + K_{M(GDP,SCS)}} \\ V_{SDH} \cdot \frac{[Suc]}{[Suc] + K_{M(Suc,SDH)}} \cdot \frac{[FAD]}{[FAD] + K_{M(FAD,SDH)}} \\ V_{FH} \cdot \frac{[Fum]}{[Fum] + K_{M(Fum,FH)}} \\ V_{FH} \cdot \frac{[Suc]}{[Suc] + K_{M(Suc,FH)}} \\ V_{MDH} \cdot \frac{[Mal]}{[Mal] + K_{M(Mal,MDH)}} \cdot \frac{[NAD^+]}{[NAD^+] + K_{M(NAD^+,MDH)}} \\ V_{MDH} \cdot \frac{[OA]}{[OA] + K_{M(OA,MDH)}} \cdot \frac{[NADH]}{[NADH] + K_{M(NADH,MDH)}} \end{pmatrix}$$

Differential equations of the citric acid cycle

$$\frac{d}{dt} \begin{pmatrix} [AcCoA] \\ [CoA] \\ [OA] \\ [Cit] \\ [ICit] \\ [\alpha KG] \\ [SucCoA] \\ [Suc] \\ [Fum] \\ [Mal] \\ [NAD^+] \\ [NADH] \\ [FAD] \\ [FADH_2] \\ [GDP] \\ [GTP] \\ [P] \\ [CO_2] \end{pmatrix} = S_{CAC} \cdot V_{CAC}$$

4.2.2 Module II: Electron transport chain

In this module electron transport chain (ETC) contains 4 enzymes. These enzymes catalyze 4 irreversible reactions (Table 4.2). That generates 9 differential equations with 9 variables. This module needs 3 inputs (NADH, FADH₂, O₂) and it gives 2 outputs (NAD⁺, FAD).

Stoichiometric matrix of the electron transport chain

$$S_{ETC} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & -1 & 0 & 0 \\ -1 & -1 & 1 & 0 \\ 1 & 1 & -1 & 0 \\ 0 & 0 & -2 & 4 \\ 0 & 0 & 2 & -4 \\ 0 & 0 & 0 & -1 \end{pmatrix}$$

Enzyme and reaction	substrate	V_{max} ($\frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$)	K_M (μM)
NADH:ubiquinon oxidoreductase (C1) $\text{NADH} + \text{H}^+ + \text{ubiquinon} \rightarrow$ $\rightarrow \text{NAD}^+ + \text{ubiquinol}$	NADH ubiquinon	nf	12 nf
Succinate dehydrogenase (C2) $\text{FADH}_2 + \text{ubiquinon} \rightarrow$ $\rightarrow \text{FAD} + \text{ubiquinol}$	FADH_2 ubiquinon	41	nf 0.5
Quinol:cytochrome-c reductase (C3) $\text{Ubiquinol} + 2 \text{ cytochrome c (ox)} \rightarrow$ $\rightarrow \text{ubiquinon} + 2 \text{ cytochrome c (red)}$	ubiquinol cytochrome c (ox)	65	1 9
Cytochrome oxidase (C4) $4 \text{ Cytochrome c (red)} + \text{O}_2 \rightarrow$ $\rightarrow 4 \text{ cytochrome c (ox)} + 2 \text{ H}_2\text{O}$	cytochrome c (red) O_2	0.51	37 1

Table 4.2: Enzymes and reactions in the electron transport chain

Reaction rate vector of the electron transport chain

$$V_{ETC} = \begin{pmatrix} V_{C1} \cdot \frac{[\text{NADH}]}{[\text{NADH}] + K_{M(\text{NADH}, C1)}} \cdot \frac{[\text{UQ}]}{[\text{UQ}] + K_{M(\text{UQ}, C1)}} \\ V_{C2} \cdot \frac{[\text{FADH}_2]}{[\text{FADH}_2] + K_{M(\text{FADH}_2, C2)}} \cdot \frac{[\text{UQ}]}{[\text{UQ}] + K_{M(\text{UQ}, C2)}} \\ V_{C3} \cdot \frac{[\text{UQH}_2]}{[\text{UQH}_2] + K_{M(\text{UQH}_2, C3)}} \cdot \left(\frac{[\text{Cyt}c(\text{ox})]}{[\text{Cyt}c(\text{ox})] + K_{M(\text{Cyt}c(\text{ox}), C3)}} \right)^2 \\ V_{C4} \cdot \frac{[\text{O}_2]}{[\text{O}_2] + K_{M(\text{O}_2, C4)}} \cdot \left(\frac{[\text{Cyt}c(\text{red})]}{[\text{Cyt}c(\text{red})] + K_{M(\text{Cyt}c(\text{red}), C4)}} \right)^4 \end{pmatrix}$$

Differential equations of the electron transport chain

$$\frac{d}{dt} \begin{pmatrix} [\text{NAD}^+] \\ [\text{NADH}] \\ [\text{FAD}] \\ [\text{FADH}_2] \\ [\text{UQ}] \\ [\text{UQH}_2] \\ [\text{Cyt}c(\text{ox})] \\ [\text{Cyt}c(\text{red})] \\ [\text{O}_2] \end{pmatrix} = S_{ETC} \cdot V_{ETC}$$

4.2.3 Module III: Malate-aspartate shuttle

In this module malate-aspartate shuttle (MAS) contains 4 enzymes and 2 transporters. Enzymes catalyze 4 reversible reactions (Table 4.3) and transporters take place in 4 transports. That generates 14 differential equations with 14 variables. This module needs 2 inputs (cytoplasmic NADH and mitochondrial NAD⁺) and it gives 2 outputs (cytoplasmic NAD⁺ and mitochondrial NADH).

Enzyme and reaction	substrate	V_{max} ($\frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$)	K_M (μM)
Malate dehydrogenase (MDH-m), mitochondrial Malate + NAD ⁺ $\rightleftharpoons$ $\rightleftharpoons$ oxaloacetate + NADH + H ⁺	malate (mito) oxaloacetate (mito) NAD ⁺ (mito) NADH (mito)	110	300 34.8 140 14.5
Malate dehydrogenase (MDH-c), cytoplasmic Malate + NAD ⁺ $\rightleftharpoons$ $\rightleftharpoons$ oxaloacetate + NADH + H ⁺	malate (cyto) oxaloacetate (cyto) NAD ⁺ (cyto) NADH (cyto)	110	300 8.3 140 20.5
Aspartate transaminase (ASAT-m), mitochondrial Oxaloacetate + glutamate $\rightleftharpoons$ $\rightleftharpoons$ aspartate + α -ketoglutarate	oxaloacetate (mito) α -ketoglutarate (mito) aspartate (mito) glutamate (mito)	22.37	nf 1750 750 nf
Aspartate transaminase (ASAT-c), cytoplasmic Oxaloacetate + glutamate $\rightleftharpoons$ $\rightleftharpoons$ aspartate + α -ketoglutarate	oxaloacetate (cyto) α -ketoglutarate (cyto) aspartate (cyto) glutamate (cyto)	48.45	nf 1250 350 nf
α -ketoglutarate transporter (α KGT) α -ketoglutarate $\rightleftharpoons$ $\rightleftharpoons$ malate	malate (mito) malate (cyto) α -ketoglutarate (mito) α -ketoglutarate (cyto)	10000 9500	710 1360 170 310
Aspartate glutamate carrier (AGC) Aspartate $\rightleftharpoons$ $\rightleftharpoons$ glutamate	aspartate (mito) aspartate (cyto) glutamate (mito) glutamate (cyto)	25.2 16.1	2800 120 3000 250

Table 4.3: Enzymes and reactions in the malate-aspartate shuttle

Stoichiometric matrix of the malate-aspartate shuttle

$$S_{MAS} = \begin{pmatrix} 1 & -1 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & -1 & 1 & 0 & 0 \\ -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 1 & -1 \\ -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & -1 & 0 & 0 \\ 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & -1 & 1 \\ 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Reaction rate vector of the malate-aspartate shuttle

$$V_{MAS} = \begin{pmatrix} V_{MDH-m} \cdot \frac{[Mal_m]}{[Mal_m] + K_{M(Mal,MDH-m)}} \cdot \frac{[NAD_m^+]}{[NAD_m^+] + K_{M(NAD^+,MDH-m)}} \\ V_{MDH-m} \cdot \frac{[OA_m]}{[OA_m] + K_{M(OA,MDH-m)}} \cdot \frac{[NADH_m]}{[NADH_m] + K_{M(NADH,MDH-m)}} \\ V_{MDH-c} \cdot \frac{[Mal_c]}{[Mal_c] + K_{M(Mal,MDH-c)}} \cdot \frac{[NAD_c^+]}{[NAD_c^+] + K_{M(NAD^+,MDH-c)}} \\ V_{MDH-c} \cdot \frac{[OA_c]}{[OA_c] + K_{M(OA,MDH-c)}} \cdot \frac{[NADH_c]}{[NADH_c] + K_{M(NADH,MDH-c)}} \\ V_{ASAT-m} \cdot \frac{[OA_m]}{[OA_m] + K_{M(OA,ASAT-m)}} \cdot \frac{[Glu_m]}{[Glu_m] + K_{M(Glu,ASAT-m)}} \\ V_{ASAT-m} \cdot \frac{[Asp_m]}{[Asp_m] + K_{M(Asp,ASAT-m)}} \cdot \frac{[\alpha KG_m]}{[\alpha KG_m] + K_{M(\alpha KG,ASAT-m)}} \\ V_{ASAT-c} \cdot \frac{[OA_c]}{[OA_c] + K_{M(OA,ASAT-c)}} \cdot \frac{[Glu_c]}{[Glu_c] + K_{M(Glu,ASAT-c)}} \\ V_{ASAT-c} \cdot \frac{[Asp_c]}{[Asp_c] + K_{M(Asp,ASAT-c)}} \cdot \frac{[\alpha KG_c]}{[\alpha KG_c] + K_{M(\alpha KG,ASAT-c)}} \\ V_{\alpha KGT} \cdot \frac{[\alpha KG_m]}{[\alpha KG_m] + K_{M(\alpha KG_m, \alpha KGT)}} \cdot \frac{[Mal_c]}{[Mal_c] + K_{M(Mal_c, \alpha KGT)}} \\ V_{\alpha KGT} \cdot \frac{[\alpha KG_c]}{[\alpha KG_c] + K_{M(\alpha KG_c, \alpha KGT)}} \cdot \frac{[Mal_m]}{[Mal_m] + K_{M(Mal_m, \alpha KGT)}} \\ V_{AGC} \cdot \frac{[Asp_m]}{[Asp_m] + K_{M(Asp_m, AGC)}} \cdot \frac{[Glu_c]}{[Glu_c] + K_{M(Glu_c, AGC)}} \\ V_{AGC} \cdot \frac{[Asp_c]}{[Asp_c] + K_{M(Asp_c, AGC)}} \cdot \frac{[Glu_m]}{[Glu_m] + K_{M(Glu_m, AGC)}} \end{pmatrix}$$

Differential equations of the malate-aspartate shuttle

$$\frac{d}{dt} \begin{pmatrix} [OA_m] \\ [\alpha KG_m] \\ [Mal_m] \\ [Asp_m] \\ [Glu_m] \\ [NAD_m^+] \\ [NADH_m] \\ [OA_c] \\ [\alpha KG_c] \\ [Mal_c] \\ [Asp_c] \\ [Glu_c] \\ [NAD_c^+] \\ [NADH_c] \end{pmatrix} = S_{MAS} \cdot V_{MAS}$$

4.2.4 Module IV: Citrate-pyruvate shuttle

In this module citrate-pyruvate shuttle (CPS) contains 7 enzymes and 3 transporters. Enzymes catalyze 5 irreversible and 2 reversible reactions and transporters take place in 6 transports (Table 4.4). That generates 24 differential equations with 24 variables. This module needs 4 inputs (cytoplasmic ATP, coenzyme A, $NADP^+$ and mitochondrial ATP) and it gives 5 outputs (cytoplasmic NADPH, acetyl-CoA, ADP and CO_2 and mitochondrial ADP). Some of the molecules (NAD^+ , NADH on both side of membrane and mitochondrial CO_2) can play a role in the module as input and output too. That depends on the actual concentrations.

Stoichiometric matrix of the citrate-pyruvate shuttle

$$S_{CPS} = \begin{pmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ -1 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & -1 & 0 & 0 & 0 & 0 & 1 & -1 \\ 0 & 0 & -1 & 1 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 1 & -1 & 0 & 0 & -1 & 1 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Reaction rate vector of the citrate-pyruvate shuttle

$$V_{CPS} = \begin{pmatrix} V_{CS} \cdot \frac{[OA_m]}{[OA_m] + K_{M(OA,CS)}} \cdot \frac{[AcCoAm]}{[AcCoAm] + K_{M(AcCoA,CS)}} \\ V_{ACS} \cdot \frac{[Cit_c]}{[Cit_c] + K_{M(Cit,ACS)}} \cdot \frac{[ATP_c]}{[ATP_c] + K_{M(ATP,ACS)}} \cdot \frac{[CoAc]}{[CoAc] + K_{M(CoA,ACS)}} \\ V_{MDH-m} \cdot \frac{[Mal_m]}{[Mal_m] + K_{M(Mal,MDH-m)}} \cdot \frac{[NAD_m^+]}{[NAD_m^+] + K_{M(NAD^+,MDH-m)}} \\ V_{MDH-m} \cdot \frac{[OA_m]}{[OA_m] + K_{M(OA,MDH-m)}} \cdot \frac{[NADH_m]}{[NADH_m] + K_{M(NADH,MDH-m)}} \\ V_{MDH-c} \cdot \frac{[Mal_c]}{[Mal_c] + K_{M(Mal,MDH-c)}} \cdot \frac{[NAD_c^+]}{[NAD_c^+] + K_{M(NAD^+,MDH-c)}} \\ V_{MDH-c} \cdot \frac{[OA_c]}{[OA_c] + K_{M(OA,MDH-c)}} \cdot \frac{[NADH_c]}{[NADH_c] + K_{M(NADH,MDH-c)}} \\ V_{ME} \cdot \frac{[Mal_c]}{[Mal_c] + K_{M(Mal,ME)}} \cdot \frac{[NADP_c^+]}{[NADP_c^+] + K_{M(NADP^+,ME)}} \\ V_{PDH} \cdot \frac{[Pyr_m]}{[Pyr_m] + K_{M(Pyr,PDH)}} \cdot \frac{[CoAm]}{[CoAm] + K_{M(CoA,PDH)}} \cdot \frac{[NAD_m^+]}{[NAD_m^+] + K_{M(NAD^+,PDH)}} \\ V_{PC} \cdot \frac{[Pyr_m]}{[Pyr_m] + K_{M(Pyr,PC)}} \cdot \frac{[CO_{2,m}]}{[CO_{2,m}] + K_{M(CO_2,PC)}} \cdot \frac{[ATP_m]}{[ATP_m] + K_{M(ATP,PC)}} \\ V_{CTP} \cdot \frac{[Cit_m]}{[Cit_m] + K_{M(Cit_m,CTP)}} \cdot \frac{[Mal_c]}{[Mal_c] + K_{M(Mal_c,CTP)}} \\ V_{CTP} \cdot \frac{[Cit_c]}{[Cit_c] + K_{M(Cit_c,CTP)}} \cdot \frac{[Mal_m]}{[Mal_m] + K_{M(Mal_m,CTP)}} \\ V_{DIC} \cdot \frac{[Mal_m]}{[Mal_m] + K_{M(Mal_m,DIC)}} \cdot \frac{[P_c]}{[P_c] + K_{M(P_c,DIC)}} \\ V_{DIC} \cdot \frac{[Mal_c]}{[Mal_c] + K_{M(Mal_c,DIC)}} \cdot \frac{[P_m]}{[P_m] + K_{M(P_m,DIC)}} \\ V_{PT} \cdot \frac{[Pyr_c]}{[Pyr_c] + K_{M(Pyr_c,PT)}} \\ V_{PT} \cdot \frac{[Pyr_m]}{[Pyr_m] + K_{M(Pyr_m,PT)}} \end{pmatrix}$$

Enzyme and reaction	substrate	V_{max} ($\frac{\mu\text{mol}}{\text{min}\cdot\text{mg}}$)	K_M (μM)
Citrate syntase (CS) Acetyl-CoA + oxaloacetate $\rightarrow$ $\rightarrow$ citrate + CoA-SH	acetyl-CoA (mito) oxaloacetate (mito)	1.88	5 5.9
ATP citrate syntase (ACS) Citrate + CoA-SH + ATP $\rightarrow$ $\rightarrow$ acetyl-CoA + oxaloacetate + ADP + PO_4^{3-}	citrate (cyto) CoA (cyto) ATP (cyto)	13.6	110 4.2 200
Malate dehydrogenase (MDH-m), mitochondrial Malate + $\text{NAD}^+ \rightleftharpoons$ $\rightleftharpoons$ oxaloacetate + NADH + H^+	malate (mito) oxaloacetate (mito) NAD ⁺ (mito) NADH (mito)	110	300 34.8 140 14.5
Malate dehydrogenase (MDH-c), cytoplasmic Malate + $\text{NAD}^+ \rightleftharpoons$ $\rightleftharpoons$ oxaloacetate + NADH + H^+	malate (cyto) oxaloacetate (cyto) NAD ⁺ (cyto) NADH (cyto)	110	300 8.3 140 20.5
Malic enzyme (ME) Malate + $\text{NADP}^+ \rightarrow$ $\rightarrow$ pyruvate + NADPH + H_+ + CO_2	malate (cyto) NADP ⁺ (cyto)	56.35	120 9.2
Pyruvate dehydrogenase (PDH) Pyruvate + CoA + $\text{NAD}^+ \rightarrow$ $\rightarrow$ acetyl-CoA + NADH + H_+ + CO_2	pyruvate (mito) CoA (mito) NAD ⁺ (mito)	5.7	0.2 4 33
Pyruvate carboxylase (PC) Pyruvate + CO_2 + ATP $\rightarrow$ $\rightarrow$ oxaloacetate + ADP + PO_4^{3-}	pyruvate (mito) CO_2 (mito) ATP (mito)	20	220 3200 220
Citrate transporter (CTP) Citrate $\rightleftharpoons$ malate	citrate (mito) citrate (cyto) malate (mito) malate (cyto)	10500 11500	27 32 60 250
Dicarboxylate carrier (DIC) Malate $\rightleftharpoons \text{PO}_4^{3-}$	malate (mito) malate (cyto) PO_4^{3-} (mito) PO_4^{3-} (cyto)	6000 6000	920 490 930 1410
Pyruvate transporter (PT) pyruvate $\rightleftharpoons$ pyruvate	pyruvate (mito) pyruvate (cyto)	114	nf 240

Table 4.4: Enzymes and reactions in the citrate-pyruvate shuttle

Differential equations of the citrate-pyruvate shuttle

$$\frac{d}{dt} \begin{pmatrix} [AcCoA_m] \\ [CoA_m] \\ [OAc_m] \\ [Cit_m] \\ [Mal_m] \\ [Pyr_m] \\ [NAD^+_m] \\ [NADH_m] \\ [ADP_m] \\ [ATP_m] \\ [P_m] \\ [CO_{2,m}] \\ [OAc_c] \\ [Cit_c] \\ [Mal_c] \\ [Pyr_c] \\ [NAD^+_c] \\ [NADH_c] \\ [NADP^+_c] \\ [NADPH_c] \\ [ADP_c] \\ [ATP_c] \\ [P_c] \\ [CO_{2,c}] \end{pmatrix} = S_{CPS} \cdot V_{CPS}$$

4.3 Integrated model

The integrated model contains 19 enzymes and 5 transporters. Enzymes catalyze 13 irreversible and 6 reversible reactions and transporters take place in 10 transports (Table 4.4). That generates 45 differential equations with 45 variables. Integrated model needs 5 inputs (cytoplasmic ATP, coenzyme A, NADP⁺ and mitochondrial ATP, GDP) and it gives 5 outputs (cytoplasmic ADP, acetyl-CoA, NADPH, CO₂ and mitochondrial ADP, GTP).

Stoichiometric matrix of the integrated model

This matrix can be built in a straightforward way using the stoichiometric matrices of the modules. Every row represents a substrate and every column represents a reaction/transport. Due to this we use $S_{INT} = S_{45 \times 35}$ matrix.

Reaction rate vector of the integrated model

This vector is the combination of the previous vectors. Every row represents a reaction/transport rate. Due to this we use $V_{INT} = V_{35}$ vector.

Differential equations of the integrated model

Differential variables vector of the integrated model (X_{INT}) is the union of the differential variables vector of the individual modules.

$$\frac{d}{dt} X_{INT} = S_{INT} \cdot V_{INT}$$

Chapter 5

Model implementation and simulation results

5.1 MATLAB platform

The mathematical model that was described before, was implemented in MATLAB platform, because it contains a lot of possibilities to solve differential equations, it is easy to code and create user interface, and the results can be plotted easily.

5.2 Solving differential equations

We have used the ode23s solver suitable for stiff problems. The solving function creates a matrix, which contains simulated concentrations of mitochondrial substrates in each time point. Parameters of differential equations (parameters of enzymes and transporters) were inserted into matrices, which were determined before the simulations. Initial values of the state variables (intramitochondrial) and constant (extramitochondrial) concentrations can be provided by the user, depending on the simulated metabolic status. Rate equations are determined for each component during simulations.

5.3 Representation

After calculating, the program creates a plot, that shows the concentrations (relative or μM) depending on time (iterations or min), between the initial and final time points. Each module has an individual plot, which represents its function.

5.4 Simulation results

5.4.1 Modules with numerical method

In this section we first test the model qualitatively (K_M and V_{max} values are 1). Parameters which were collected partially from the available literature and also determined from laboratory measurements, are going to be built later in the model, because for this process we need suitable mitochondrial and cytoplasmic concentrations to test the result. The aim is only to obtain a rough description of the dynamical behavior of the overall system. However we used simplified equation, the results are meaningful. The results can be seen in the subsections. Every module gets individual initial values and suitable inputs and gives us outputs.

5.4.2 Citric acid cycle

Simulation of the first module is the simulation of the citric acid cycle. Initial values are 0.5 (acetyl-CoA, oxaloacetate, NAD, FAD, GDP, $H_2PO_4^-$, CoA-SH) or zero (other components). We supply the system in every cycle with acetyl-CoA, NAD^+ , FAD, GDP, $H_2PO_4^-$ (1:3:1:1:1 ratio). In the results we can see, that the components of citric acid cycle reach steady state (Figure 5.1). Other components can be seen in (Figure 5.2). NAD^+ , FAD, GDP and $H_2PO_4^-$ (similar value as GDP) reach steady state, and other molecules (NADH, $FADH_2$, GTP and CO_2) increase because these are the outputs of this module.

5.4.3 Electron transport chain

Simulation of the second module is the simulation of the electron transport chain. Initial values are 1 (ubiquinon, cytochrome c (ox), O_2), zero (NAD^+ , FAD, ubiquinol, cytochrome c (red)), 0.25 ($FADH_2$) or 0.75 (NADH). We supply the system in every cycle with NADH, $FADH_2$ and O_2 (3:1:2 ratio). Results show us, that the components of electron transport chain reach steady state and the 2 outputs of this module (NAD^+ , FAD) increase (Figure 5.3). We can observe, that concentration sum of the participants of the chain ($UQ + UQH_2$ and $Cytc(ox) + Cytc(red)$) are constant, because these molecules change only their redox status. In this case plots of these molecules are reciprocal. If we eliminate the outputs (NADH and $FADH_2$) in every cycle, sum of the reduced equivalents ($NAD^+ + NADH$ and $FAD + FADH_2$), have similar behavior (Figure 5.4).

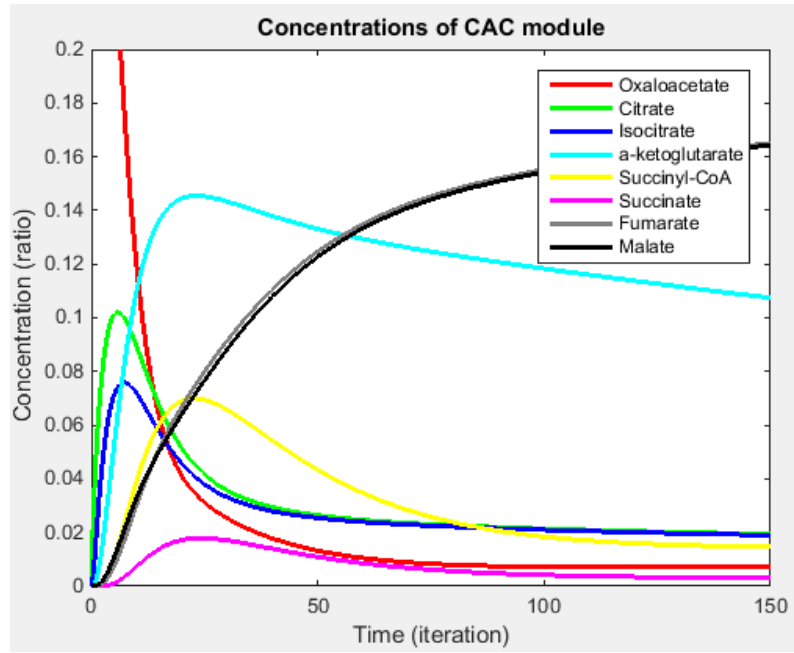


Figure 5.1: Concentrations of citric acid cycle molecules

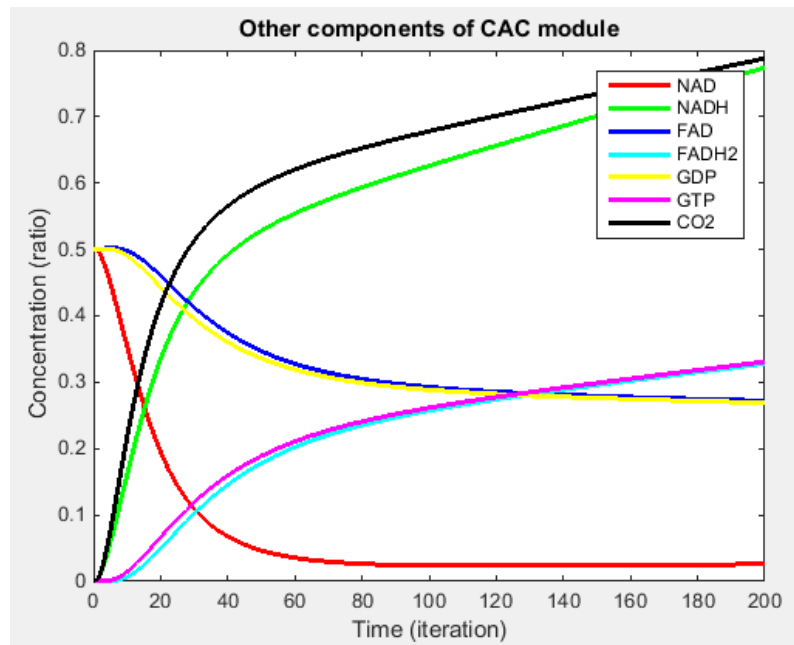


Figure 5.2: Other components of citric acid cycle module

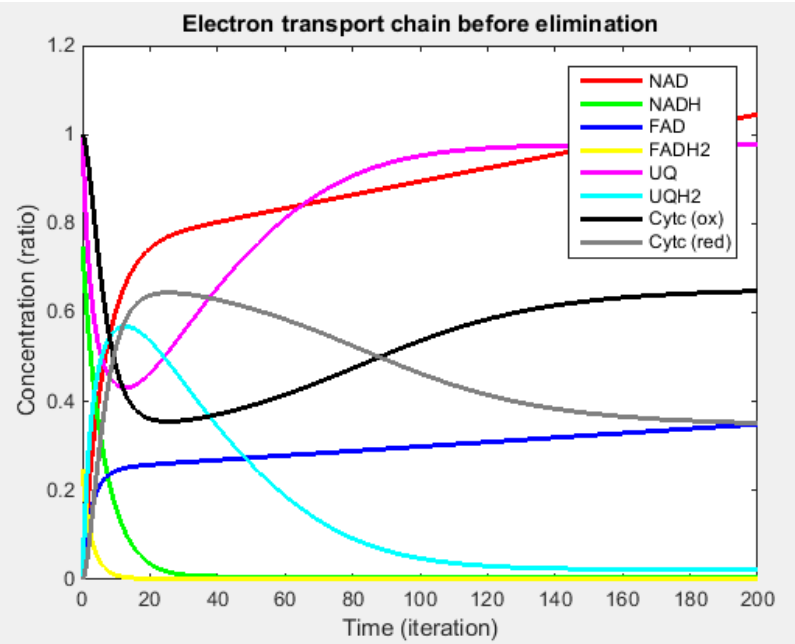


Figure 5.3: Concentrations of ETC before elimination

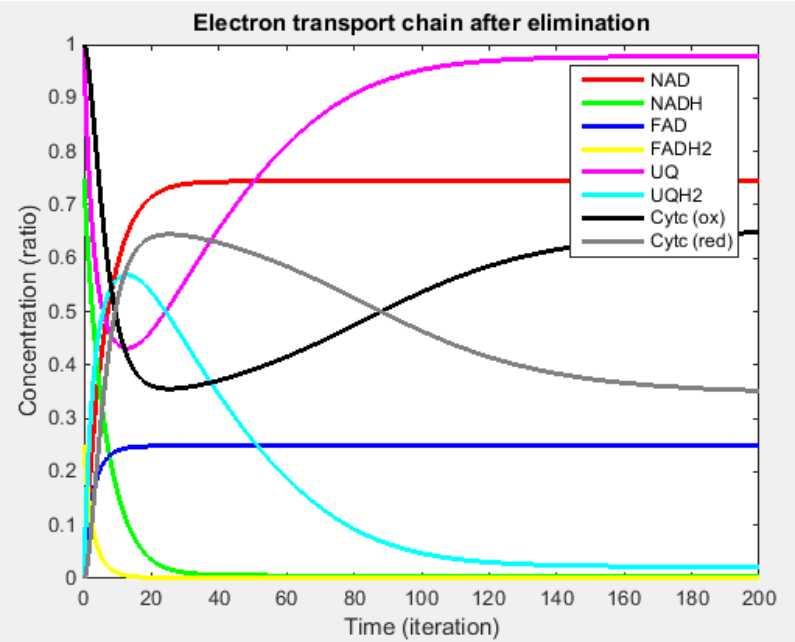


Figure 5.4: Concentrations of ETC after elimination

5.4.4 Malate-aspartate shuttle

Simulation of the third module is the simulation of the malate-aspartate shuttle. Initial values in mitochondrion: oxaloacetate is 0.5, α -ketoglutarate, malate and NAD^+ are zero, glutamate and aspartate are 0.25, and NADH is 0.75. Initial values in cytoplasm are 0.2 except NAD^+ (0) and NADH (0.5). We supply the system in every cycle with mitochondrial NAD^+ and cytosolic NADH (1:1 ratio) and we eliminate the outputs (mitochondrial NADH and cytosolic NAD^+ , 1:1 ratio). Results show us, that the components of malate-aspartate shuttle reach steady state both in mitochondrion (Figure 5.5) and cytoplasm (Figure 5.6). This simulation shows that the model can produce NAD^+/NADH equilibrium status between the two sides of the inner membrane.

5.4.5 Citrate-pyruvate shuttle

Simulation of the fourth module is the simulation of the citrate-pyruvate shuttle. Initial values in the matrix: acetyl-CoA, CoA-SH, oxaloacetate, pyruvate, ATP and PO_4^{3-} are 0.5, NADH is 0.75, and other components are zero. Initial values in cytoplasm: CoA-SH, oxaloacetate, NADH, NADP, ATP and PO_4^{3-} are 0.5, citrate and malate are 0.2, and other components are zero. We supply the system in every cycle with cytoplasmic coenzyme A, ATP, NADP^+ and mitochondrial ATP. We eliminate cytoplasmic acetyl-CoA, ADP, NADPH, CO_2 and mitochondrial ADP (1:1 ratio). Molecules which can take role as input and output too (NAD^+ , NADH on both sides of membrane and mitochondrial CO_2) are not changed. Results show us, that this system cannot operate alone. The components don't reach steady state in the matrix (Figure 5.7) and in the cytoplasm (Figure 5.8), because in every cycle the system loses 3 carbon atoms (two with acetyl-CoA, and one with CO_2). Some of the components have constant amounts. In the mitochondrion NAD^+/NADH and ADP-ATP (Figure 5.9), in the cytoplasm NAD^+/NADH , $\text{NADP}^+/\text{NADPH}$ and ADP-ATP (Figure 5.9) show reciprocal behavior.

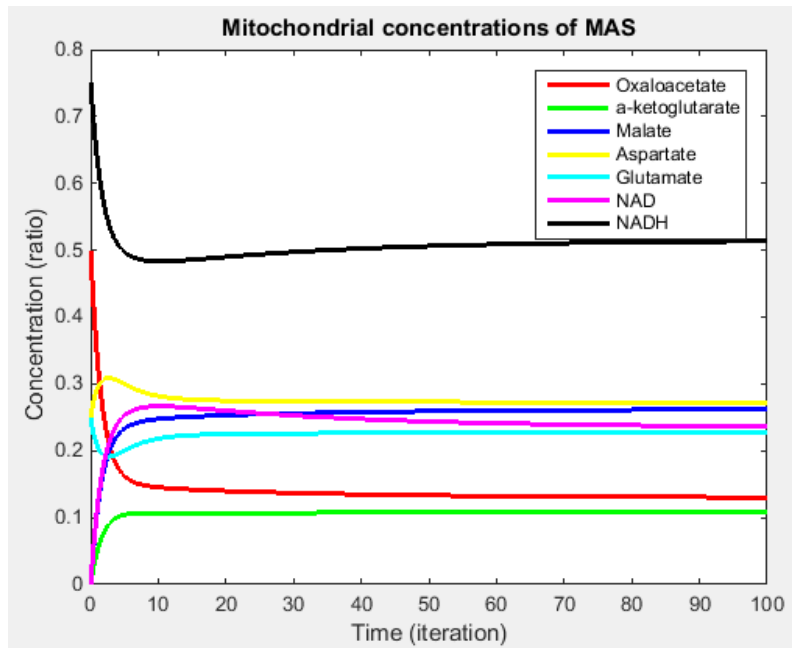


Figure 5.5: Mitochondrial concentrations of MAS

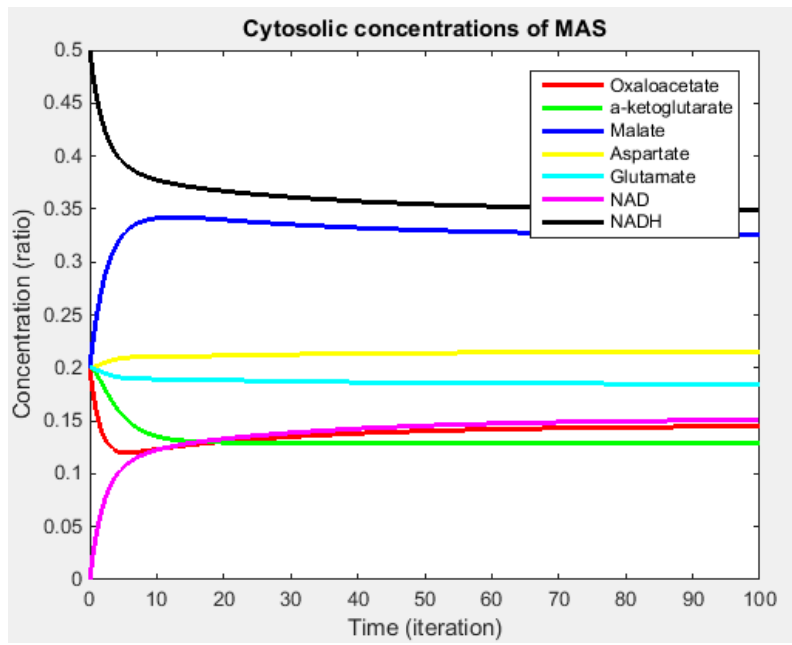


Figure 5.6: Cytosolic concentrations of MAS

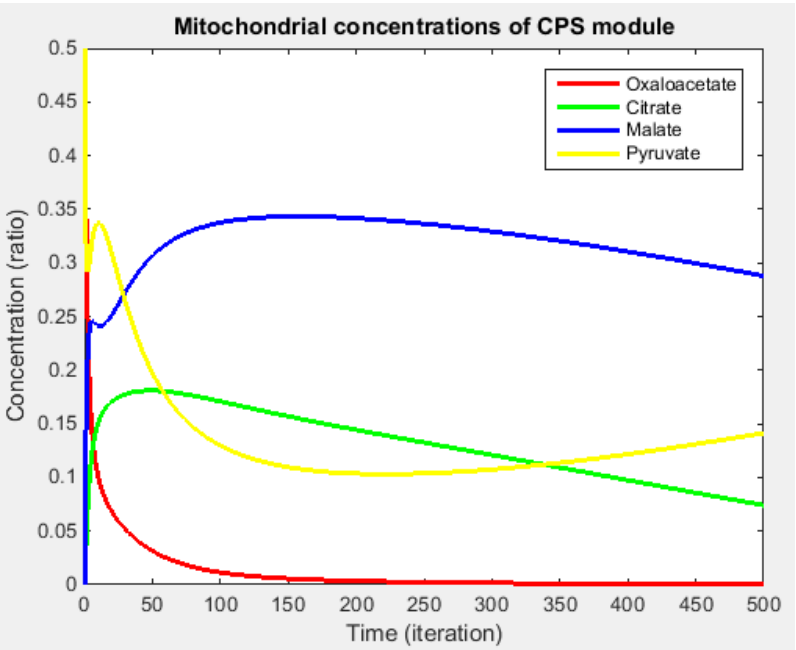


Figure 5.7: Concentrations of citric acid cycle molecules

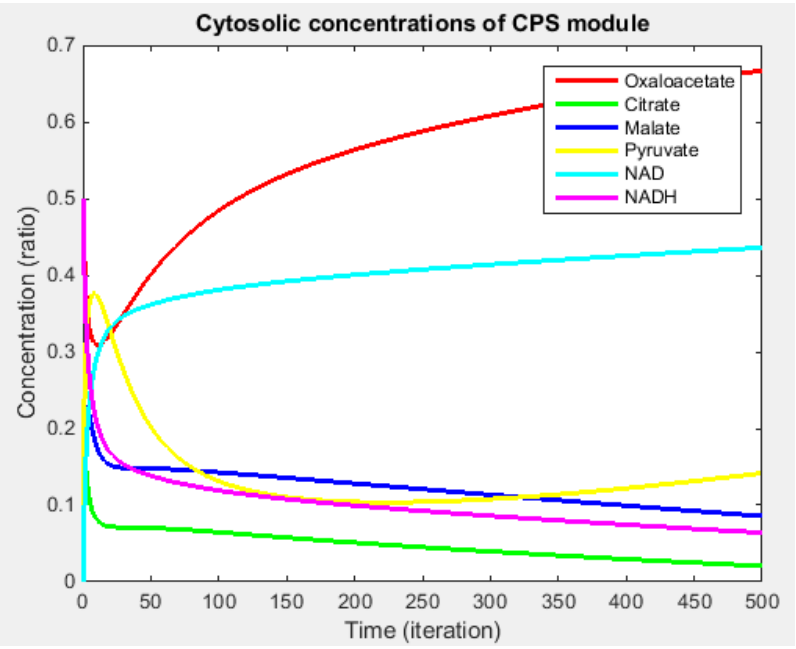


Figure 5.8: Concentrations of citric acid cycle molecules

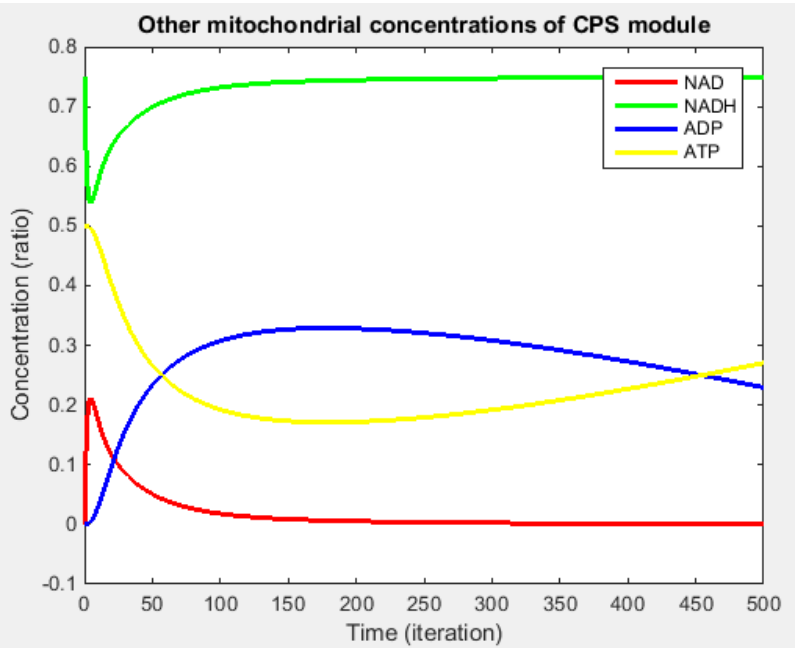


Figure 5.9: Concentrations of citric acid cycle molecules

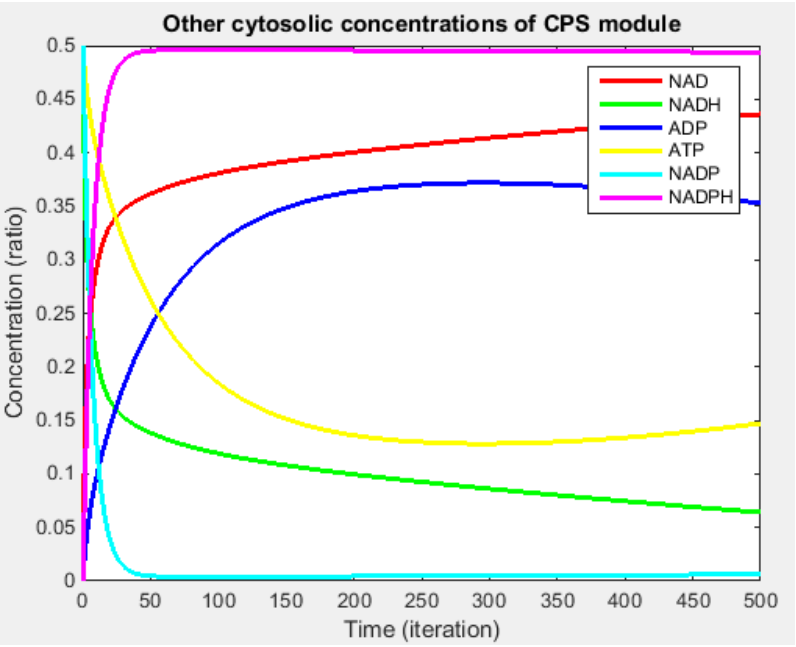


Figure 5.10: Concentrations of citric acid cycle molecules

5.5 Integrated model

In this subsection, we present simulation results of the integrated model. Initial values in the matrix are same as in the previous simulations: acetyl-CoA, CoA-SH, oxaloacetate, pyruvate, ATP and PO_4^{3-} are 0.5, aspartate, glutamate and FADH_2 are 0.25, NADH is 0.75, ubiquinon, cytochrome c and O_2 are 1, and other concentrations are zero. Initial values in cytoplasm: CoA-SH, oxaloacetate, NADH, NADP, ATP and PO_4^{3-} are 0.5, citrate, α -ketoglutarate, malate, aspartate and glutamate are 0.2, and other components are zero. We supply the system in every cycle with cytoplasmic coenzyme A, ATP, NADP^+ and mitochondrial ATP and GDP. We eliminate cytoplasmic acetyl-CoA, ADP, NADPH, CO_2 and mitochondrial ADP and GTP (1:1 ratio). Molecules which can take role as input and output too (NAD^+ , NADH in both side of membrane and mitochondrial CO_2) are not changed. The results show that the dynamical behavior of the integrated model differs in several characteristics from the individual models.

The first comparison is between the components of the citric acid cycle. Integrated model shows, that all of the molecules reach steady state, because it isn't an isolated system. Most of the components have at least one alternative pathway, which they can use, and due to this we can see quick equilibration by these molecules (Figure 5.11).

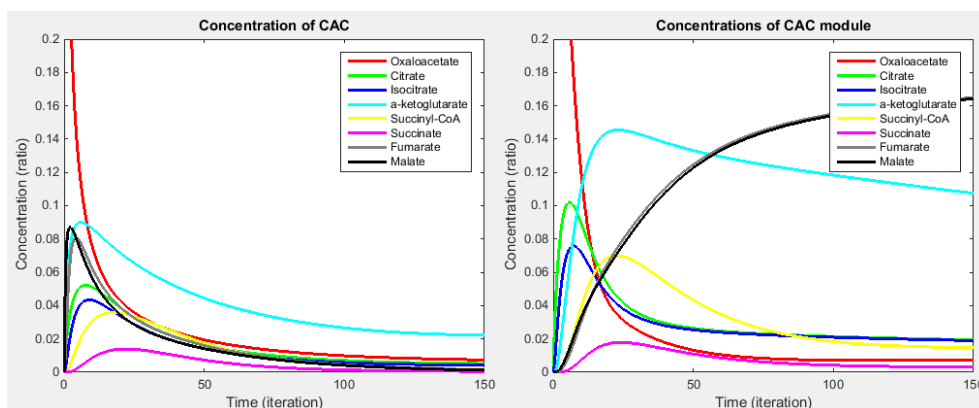


Figure 5.11: Concentrations of citric acid cycle molecules

The second comparison is between the components of the electron transport chain. This module connects to citric acid cycle via reduced equivalents. NADH and FADH_2 are outputs of citric acid cycle and inputs of the electron transport chain. NAD^+ and FAD are outputs of the electron transport chain and inputs of citric acid cycle. We assume, that all of the NADH and FADH_2

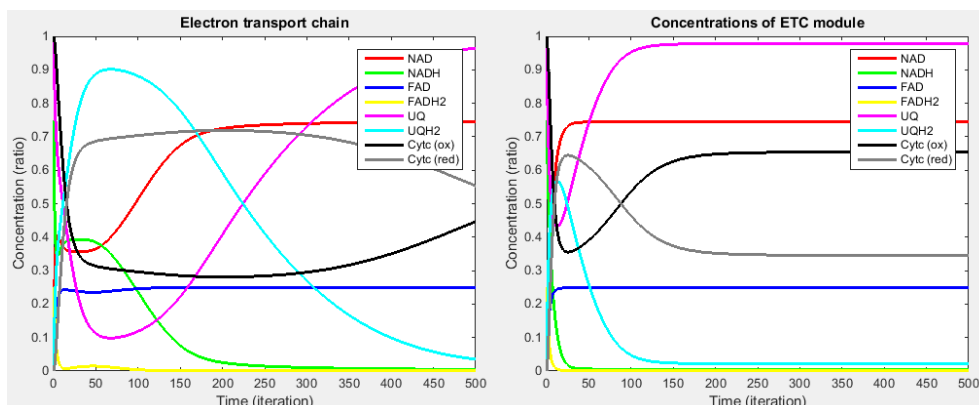


Figure 5.12: Concentrations of ETC molecules

are oxidized via electron transport chain. Because of this we don't use these molecules as input and output in the integrated model. In this comparison we can see, that the integrated model needs more time to reach steady state in electron transport chain molecules, because of these system is relatively separate from others (Figure 5.12).

The third comparison is between the mitochondrial components of the malate-aspartate shuttle. This module connects to citric acid cycle via mitochondrial malate, oxaloacetate, NAD^+ and NADH , because these molecules take part of both process. Results show us, that the connection modifies the function of malate-aspartate shuttle. NADH can be oxidized in the electron transport chain and decreases its amount, due to this the $\text{malate} + \text{NAD}^+ \rightleftharpoons \text{oxaloacetate} + \text{NADH}$ reaction becomes faster in the right direction, and because of this the amount of malate is lower than in the individual model. In other case, if the intramitochondrial malate is low, the α -ketoglutarate transporter functions rapidly. It carries more malate from the cytoplasm to the mitochondrial matrix and carries α -ketoglutarate to reverse direction. Due to this the intramitochondrial α -ketoglutarate decreases. In the integrated model, aspartate and glutamate concentrations in the matrix show reciprocal behavior because of the aspartate aminotransferase reaction (Figure 5.13).

In the comparison of the cytoplasmic concentration we recognize the totally different concentrations. Amount of malate decreases, because of the α -ketoglutarate transporter as we mentioned. Due to this $\text{malate} + \text{NAD}^+ \rightleftharpoons \text{oxaloacetate} + \text{NADH}$ reaction go to left direction, which results that the concentration of oxaloacetate decreases. Because of this the system tries to restore equilibrium state, produces oxaloacetate (and glutamate) from aspartate and (transported) α -ketoglutarate. Due to this concentrations change:

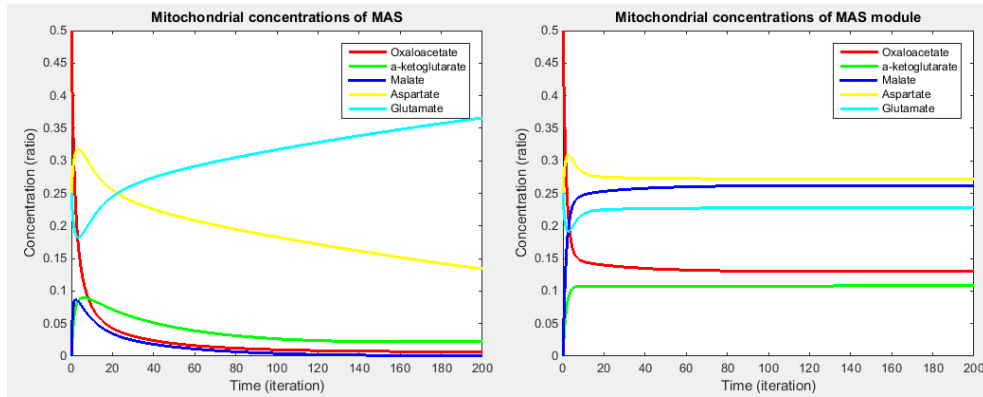


Figure 5.13: Concentrations of MAS molecules in the matrix

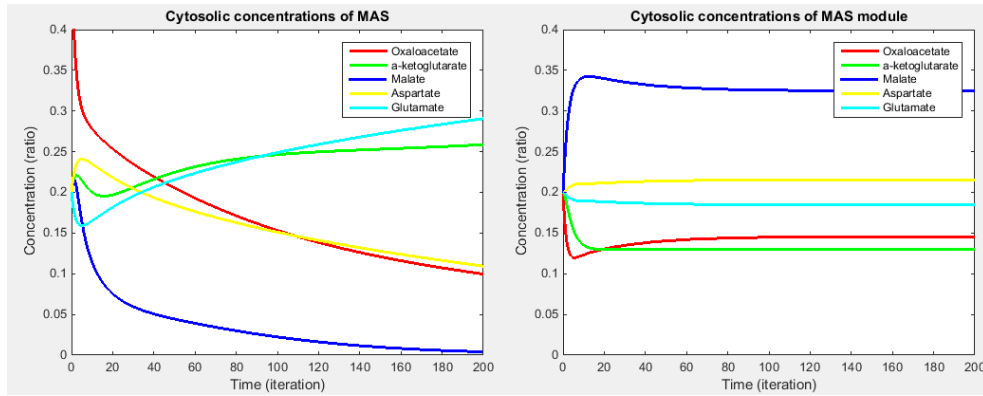


Figure 5.14: Concentrations of MAS molecules in the cytoplasm

α -ketoglutarate reaches steady state, glutamate increases, oxaloacetate and aspartate decrease (Figure 5.14).

The fifth comparison is between the mitochondrial components of the citrate-pyruvate shuttle. This module connects to citric acid cycle via mitochondrial citrate, malate, oxaloacetate, acetyl-CoA, Coenzyme A, NAD⁺ and NADH. These molecules take part in both processes. Because of the concentration differences: intramitochondrial malate is lower in the integrated model, because the malate dehydrogenase reaction takes part in each modules and it is a substrate three transporter. Due to this its amount decreases quickly. Citrate concentration in the matrix is lower too, because it has an other pathway to react via aconitase. Pyruvate concentration is higher than in the individual module, because in the module concentration of malate is higher, this leads to more oxaloacetate, which eliminates more acetyl-CoA,

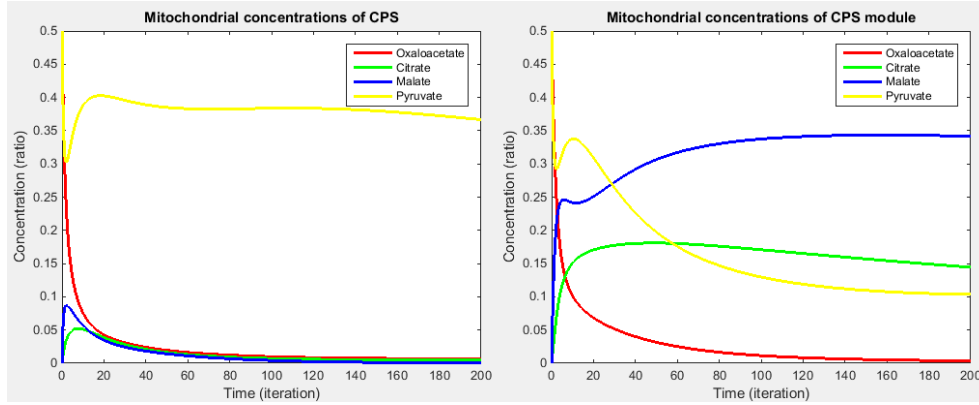


Figure 5.15: Concentrations of CPS molecules in the matrix

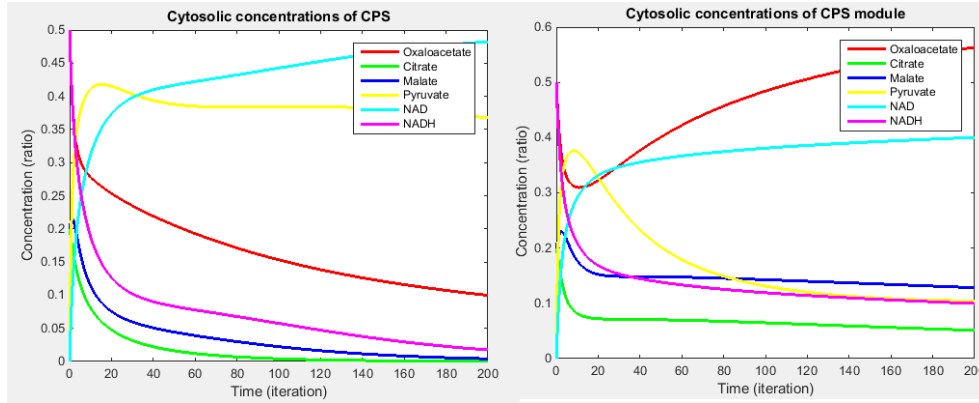


Figure 5.16: Concentrations of CPS molecules in the cytoplasm

which is produced from pyruvate via pyruvate dehydrogenase (Figure 5.15).

The last comparison is about the cytoplasmic concentration of the citrate-pyruvate shuttle's molecules. Because of the different oxaloacetate concentrations malate concentration is low as we mentioned in the fourth comparison. In this module an other pathway can eliminate malate via malic enzyme. Pyruvate concentration is the same as in the matrix, because of pyruvate transporter, which transports pyruvate in the direction of concentration gradient. Amount of citrate is lower than in the individual modul because the intramitochondrial concentration of citrate is lower, because the aconitase reaction, as it inhibits the transport (Figure 5.16).

Chapter 6

Conclusion

6.1 The model

During this work we set up dynamic model for describing the dynamics of key quantities in mitochondrial metabolism. This mathematical model was written in the form of 45 nonlinear ordinary differential equations using Michaelis Menten kinetics. Reversible reactions were modeled as two opposite elementary reactions. This model is different from previous constructions in the literature where models were typically simplified with reduction methods or ratios of parameters ($\frac{v}{v_{max}}, \frac{[S]}{K_M}$). The greatest model which we have founded in the literature used 29 differential equations to describe this system [6]. Parameters which were collected partially from the available literature and also determined from laboratory measurements taken at the Institute of Medical Biochemistry, Semmelweis University, Budapest. The model is implemented in MATLAB platform.

6.2 Evaluation of the simulation results

Simulation shows that the obtained results are qualitatively acceptable for our expectations. On the other hand in some cases huge inaccuracies were experienced. We should take into collected parameters and consideration the metabolic properties of these substances and later incorporate that into the model, so we can get more accurate results. Additional effects of other substrates which influence functions of enzymes and transporters should also be built into the model.

Further work will be focused on improving the accuracy of the model by involving more transporters which can make our system more physiological, because the modeling of the connection between the mitochondrion and its

environment is more precise. Some substances are usable as inhibitors for enzymes and transporters (K_i) it would be practical to build in these effects to the model. New metabolic pathways that are connected to citric acid cycle or its anaplerotic reactions can help us to make our modeling and reaction dynamic more accurate. We would like to measure parameter values of mitochondrial enzymes at similar conditions (K_M and V_{max}), because in the literature we did not find coherent data for that. Additionally we would like to simulate modified function and equilibrium states of citric acid cycle which is caused one or more mutant enzymes or transporters. This can be modeled if we change the parameters of enzymes or transporters. With these parameters we can study mitochondria which have enzyme or transporter mutations. We can improve the accuracy of the model by applying efficient parameter estimation techniques together with sensitivity and identifiability analysis.

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